AWARD NUMBER: W81XWH-10-1-0910

TITLE: SCI with Brain Injury: Bedside to Bench Modeling For Developing Treatment and Rehabilitation Strategies

PRINCIPAL INVESTIGATOR: Michael S. Beattie, Ph.D

CONTRACTING ORGANIZATION: University of California, San Francisco San Francisco, CA 94103

REPORT DATE: October 2013

TYPE OF REPORT: Final

PREPARED FOR: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE: October 2013	2. REPORT TYPE	3. DATES COVERED
	Final	30 Sep 2010 - 29 Sep 2013

4. TITLE AND SUBTITLE

SCI with Brain Injury: Bedside to Bench Modeling For Developing Treatment And Rehabilitation Strategies

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0910

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Michael S. Beattie PhD

E-Mail: Michael.Beattie@ucsf.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Regents of the University of California, San Francisco
San Francisco, CA 94103

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Spinal cord injury (SCI) is often accompanied by traumatic brain injury (TBI), but evidence-based approaches for treatment of this "dual-diagnosis" are lacking. We used clinical-practice evidence to guide development of an animal model for studying the dual injury to open new directions for therapy. We made an animal model that showed unexpected interactions between these injuries. We then built a clinical TBI+SCI patient database from San Francisco General Hospital, the Santa Clara Valley Medical Center and from the VA Palo Alto Health Care System, covering acute and chronic stages of recovery. Common data elements were made for querying patient records, resulting in an overview of recovery and showing that more medications are used in the dual-diagnosis patients than either injury alone. Agents tested in the new model showed that drug effects differ with injury type. The project has resulted in a continuing community of practice and research, and the animal model should continue to inform and be informed by the clinical enterprise.

15. SUBJECT TERMS
spinal cord injury, traumatic brain injury, dual diagnosis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	49	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

INTRODUCTION

The goal of this Translational Research Partnership was to gather information on clinical outcomes and practices from several collaborating neurotrauma programs and synthesize this information to inform the development of relevant animal models of the dual diagnosis of Spinal Cord Injury (SCI) and Traumatic Brain Injury (TBI). These models are being used to identify improved therapeutic strategies that can be tested in the clinical setting. The process is meant to be iterative and interactive, producing a "community of practice and research."

The project links the Brain and Spinal Injury Center at the University of California, San Francisco (UCSF) with the Spinal Cord Injury and Brain Injury units at the Santa Clara Valley Medical Center (SCVMC) and the VA Palo Alto Health Care System (VAPAHCS).

During the project, a teleconferencing system was established to facilitate communication among the study sites, and all Investigators participated in a series of meetings which rotated between participating medical centers to develop clinical database search strategies and information dissemination during the project. The search strategies allowed us to synthesize a dual diagnosis database, which is reported in more detail below.

A dual injury model in rats was developed and initial drug testing was accomplished. It was learned that dual injuries produce complicated outcomes depending on the site of injury and that treatments that improve recovery after spinal cord injury do not necessarily promote recovery after brain injury.

BODY

In the text below, we have taken the Statement of Work as a template and detailed how each aim was completed and milestones accomplished.

Specific Aim 1: Develop community of practice and research and focus groups; and develop clinical database search strategy and dual diagnosis data

Task 1: Continue development of community of practice and research and focus groups

1a. All Investigators' meetings

The Principal and Partnering Investigators have continued to meet to develop the community of practice and research linking the basic scientists working on animal models of brain and spinal cord injury with the clinical scientists working with patients having spinal cord injuries and traumatic brain injuries. During the second year of the project, this community has continued to develop and has grown to include several new members. Face-to-face meetings have been held at the VAPAHCS and UCSF as described below.

1b. Teleconference set up

Telephone and internet-based audio conferencing has been set up to facilitate collaboration and reduce the amount of time spent in traveling between institutions in different parts of the Bay Area. WebEx software has been used to allow multicast audio.

1c. Teleconferences

Telephone conferences have been held twice a month on average (see KEY RESEARCH ACCOMPLISHMENTS).

Milestones:

Focus Groups (FG) have continued to be conducted at major national and international conferences.

- Combined conference of International Spinal Cord Society and American Spinal Injuries Association, Washington, DC, 2011

Meeting of Drs. Beattie, Creasey and McKenna with Dr. Fin Biering-Sorensen, President of International Spinal Cord Society, regarding Common Data Elements which he has championed internationally. Focus group with hand therapists from Cleveland Ohio and VA Palo Alto. Participation in symposium on Common Data Elements.

- American Spinal Injuries Association, Denver, Colorado 2012

Meeting of Drs. Beattie, McKenna and Creasey with Dr. Sukvinder Kalsi-Ryan who developed the Graded and Redefined Assessment of Strength, Sensibility and Prehension (GRASSP) for assessment of hand function, and Lisa Johansen, PhD, RPT, who is using it at VAPAHCS.

- International Spinal Cord Society, London, England, 2012
- Stanford Symposium on Regeneration, Repair and Restoration of Function after Spinal Cord Injury, November 16-17 2012.

- VAPAHCS SCI+TBI Research Forum meetings – March 16, 2012, and March 15, 2013. Presentations by partnering PI, Graham Creasey (2012) and poster presentations describing consensus reports (Inoue et al., 2012 and Guandique et al., 2013) were also made.

Task 2: Develop clinical database search strategy

Developing a clinical database search strategy was accomplished in year one of the grant. Access to the databases and review of the charts continued during year two and into year 3. The information desired was difficult to obtain and it was necessary to establish and a number of approaches were used. Individual chart review, text mining software for content analysis, attempts to run principal component analysis, were all used to evaluate the records. The data available covered different time spans post-injury, so comparing details across the three sites was difficult. Reviews of individual patient charts to identify medications used at the time of initiation of rehabilitation and at the time of discharge from acute rehabilitation were done. These trends in medication use and discontinuation were provided to the animal model group in an effort to model clinical practice from the bedside to bench.

Task 3: Query Dual Diagnosis clinical database

The investigators obtained data from the SCI and the TBI Systems of Care at SCVMC, San Francisco General Hospital, and the SCI Service and the Polytrauma Center at VAPAHCS.

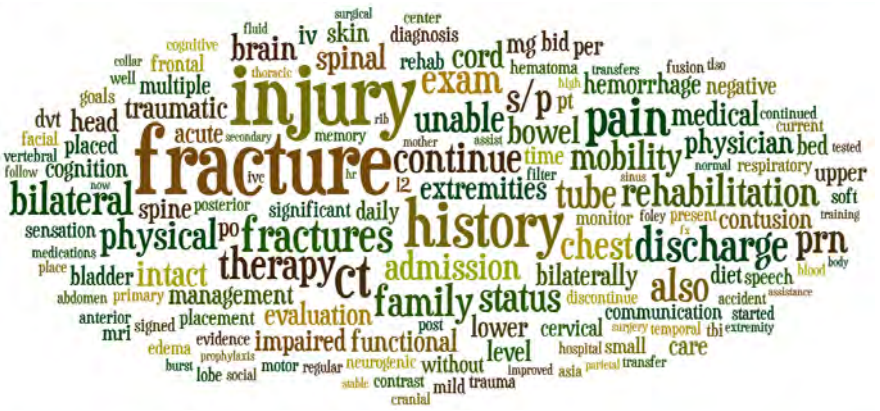
3a1. SCI and TBI Systems of Care at SCVMC

Patients who had undergone TBI rehabilitation at SCVMC between 1989 and 2010 were identified in the TBI Model Systems (TBIMS) National Database when the Form I (enrollment data) also indicated the presence of an SCI.

The admission and discharge notes of patients (n=14) with combined TBI and SCI diagnosis were then extracted from the hospital records of SCVMC and text mined in detail. The text was parsed into a database that was designed to track the treatment and recovery of the patients throughout the time spent in the brain and spinal rehabilitation facility. Once the database was compiled, an initial comparison utilizing a word cloud analysis of admission and discharge notes was used to provide the first broad visualization of the database.

In this word cloud analysis, the larger a word is in size, the more frequently it appears in the document. In the example below, “fracture” appeared in high frequency. We determined that many of the TBI and SCI patients had undergone multiple fractures at the time of their injury. This was in keeping the expectation that TBI and SCI patients experience multiple co-morbidities or “polytrauma.”

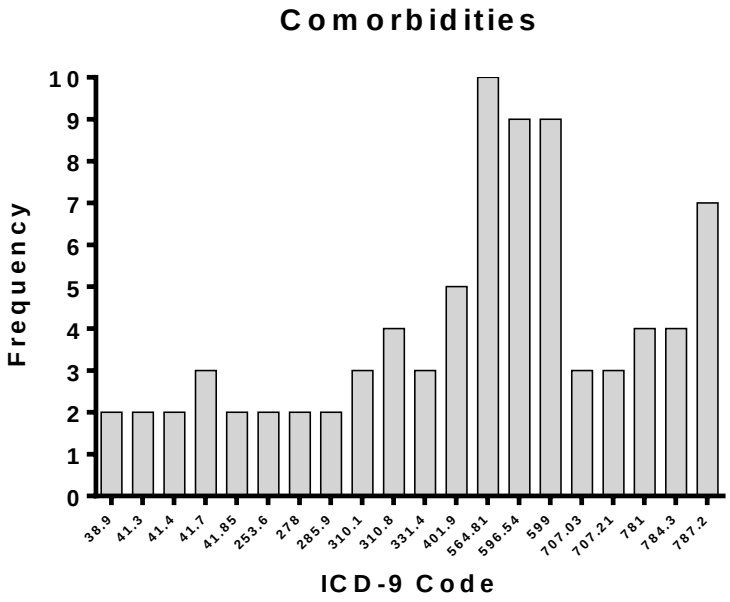
Admission notes:



Discharge notes:

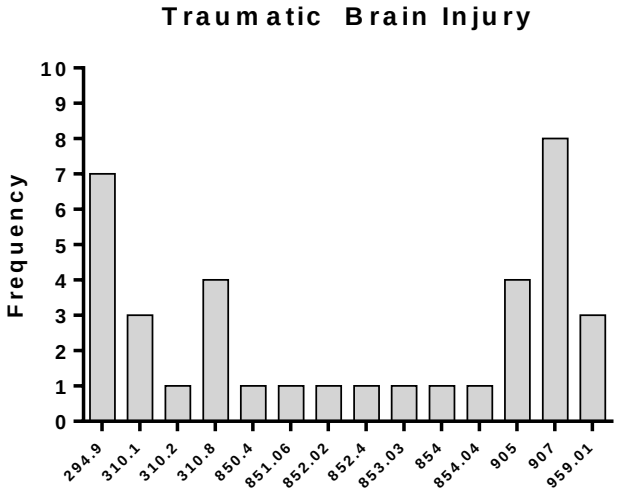
The patient cohort with SCI+TBI had a total of 343 unique International Classification of Diseases, Ninth Edition (ICD-9) codes, confirming the variety of comorbidities in this sample. A histogram of the twenty commonest ICD-9 codes for co-morbidities is shown

below.

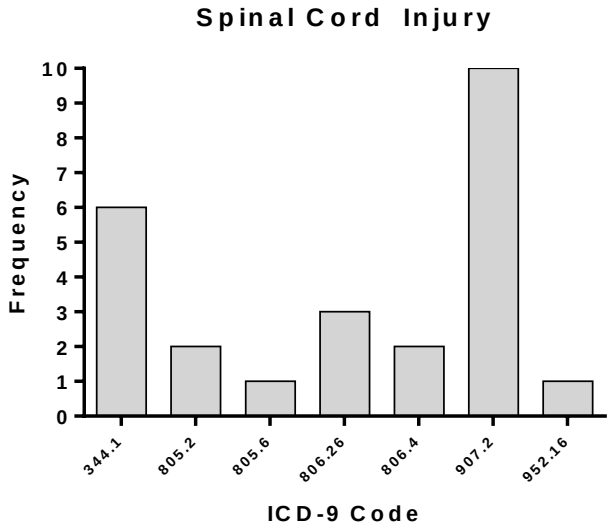


Type	Code	Subject(s)
SEPTICEMIA NOS	38.9	1, 8
K. PNEUMONIAE INFECT	41.3	13, 14
E. COLI INFECTION	41.4	1, 14
PSEUDOMONAS INFECT NOS	41.7	8, 9, 12
GRAM-NEG BACT INFECT NEC	41.85	4, 13
NEUROHYPOPH DISORD NEC	253.6	4, 8
OBESITY NOS	278	4, 9
ANEMIA NOS	285.9	3, 12
PERSONALITY CHANGE CCE	310.1	1, 7, 14
OTH NPMD FOLLOWING OBD	310.8	1,2, 8, 10
OBSTR HYDROCEPHALUS	331.4	1, 4, 8
HYPERTENSION NOS	401.9	1, 4, 8, 11, 13
NEUROGENIC BOWEL	564.81	1, 4, 7, 8, 9, 10, 11, 12, 13, 14
NEUROGENIC BLADDER NOS	596.54	1, 4, 7, 8, 9, 11, 12, 13, 14
URINARY TRACT INF NOS	599	1, 4, 7, 8, 9, 10, 12, 13, 14
PRESSURE ULCER-LOW BACK	707.03	4, 9, 14
STAGE I PRESSURE ULCER	707.21	8, 12, 14
ABN INVOL MOVEMENT NEC	781	3, 8, 12, 14
APHASIA	784.3	8, 11, 12, 13
DYSPHAGIA	787.2	1, 4, 8, 9, 11, 12, 13

ICD-9 codes commonly associated with TBI and SCI respectively were extracted from the hospital records and are shown in the two charts below.



Type	Code	Subject(s)
PERSIST MENT DIS CCE NOS	294.9	1, 3, 4, 6, 8, 12, 13
PERSONALITY CHANGE CCE	310.1	1, 7, 14
POSTCONCUSSION SYNDROME	310.2	1
OTH NPMD FOLLOWING OBD	310.8	1, 2, 8, 10
CONCUSSION-DEEP COMA	850.4	6
CORTEX CONTUSION-NEC	851.06	2
TRAUMATIC SAH-BRIEF	852.02	1
TRAUMATIC EXDH-NOS	852.4	8
OTH TRAUM ICH-MOD	853.03	11
OTH INTRACRANIAL INJURY-NOS	854	4
OTH INTRACRANIAL INJURY-LONG	854.04	6
LATE EFF SKULL/FACE FX	905	8, 11, 12, 13
LATE EFF IC INJURY	907	1, 2, 3, 4, 6, 8, 10, 14
HEAD INJURY NOS	959.01	1, 7, 10



Type	Code	Subject(s)
PARAPLEGIA	344.1	7, 9, 10, 12, 13, 14
FX DORSAL VERTEBRA-CLOSE	805.2	9, 13
FX SACRUM/COCCYX-CLOSED	805.6	1
T7-T12 FX-CL/COMP CRD	806.26	4, 9, 14
CL LUMBAR FX W CORD INJ	806.4	6, 10
LATE EFF SPINAL CORD INJ	907.2	1, 2, 3, 4, 7, 9, 10, 12, 13, 14
T7-T12 COMPL CORD LESION	952.16	7

Patients were coded with a variety of ICD-9 codes, and individual patients were often coded with more than one ICD-9 code for their SCI and also for their TBI. Note that only 13 out of 14 patients had an ICD-9 code that referenced a traumatic brain injury, and only 11 out of 14 patients had an ICD-9 code that referenced a spinal cord injury.

This indicates that using ICD-9 codes to search hospital records for patients with TBI, SCI, and both TBI and SCI is not a sufficient search strategy to identify all such patients. The search strategy actually used to identify these patients identified individuals who would have not have been found merely by searching for appropriate ICD-9 codes.

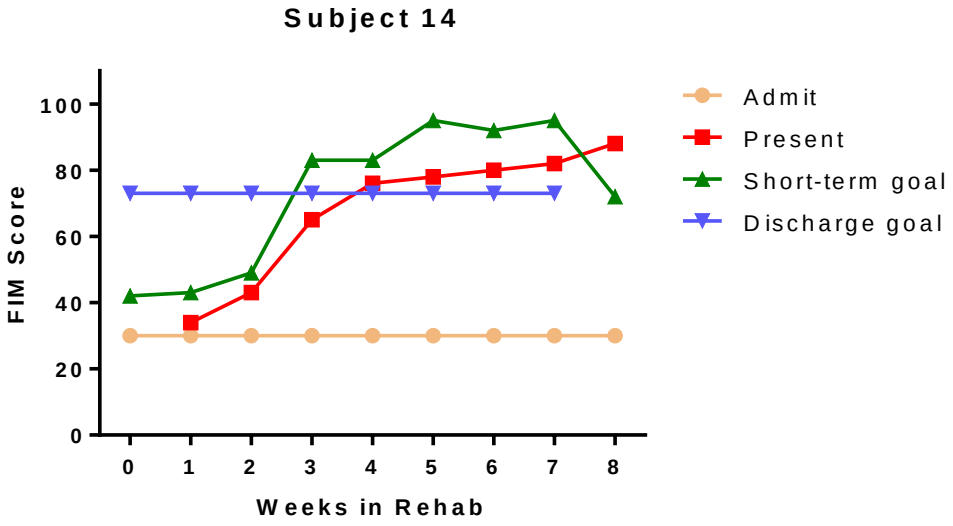
We extracted demographic data of the TBI and SCI patients in the database to provide a targeted analysis that would provide essential information for further hypothesis testing. The analysis below includes age, gender, etiology of injury, number of days until admission into rehab, length of stay in rehabilitation, and Glasgow Coma Scale (GCS). In addition, the database includes data on injury specifics, surgical procedures, diagnostic findings, physical and neurological exam findings, and rehabilitation assessment plans.

Demo-graphic	**Age**		**Gender**		**Etiology**		**# days until rehab admission**		**Rehab LOS**	
Data	Mean years	SD	% Male	% Female	%Vehicular	% Fall	Mean days	SD	Mean days	SD
TBI/SCI (N =14)	33.6	17.1	71	28.6	78.6	21.4	29.6	24.4	55.9	39.7

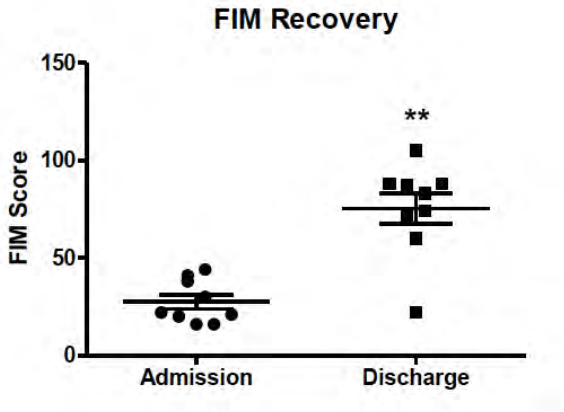
Glasgow Coma Scale	**Mild (13-15)**	**Moderate (9-12)**	**Severe (<= 8)**	**Not Reported**
TBI/SCI (N = 14)	14.29%	7.1%	64.3%	14.3%

In addition, we obtained de-identified Functional Independence Measure (FIM) scores that tracked the functional recovery of the TBI

and SCI patients during their stay in rehabilitation. FIM graphs were plotted against the number of weeks spent in rehabilitation. We were able to visualize the functional recovery of each patient throughout rehabilitation. For example, the analysis below shows a single patient with a dual TBI and SCI.



TBI/SCI Patients Improve in Functional Independence During Length of Stay in Rehabilitation.



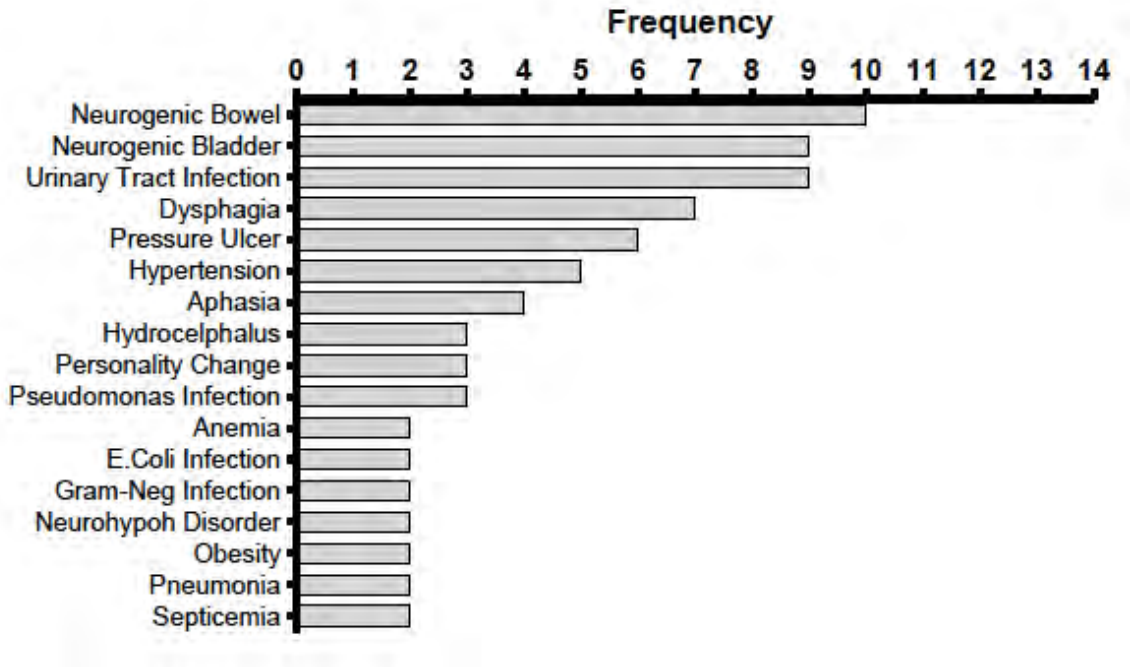
In addition to the various measures included in the database, the majority of the patients (n=9) were also assessed for functional independence at the time of admission and discharge using the FIM All of the patients assessed on the FIM showed low total FIM scores at the time of admission into the rehab facility (27.6 ± 11.0). By the time patients were discharged from rehab (43.1 ± 30.1 days), all but 1 patient had substantial recovery in their FIM scores (75.4 ± 23.7). Unfortunately we did not have any data regarding injury severity for either TBI or SCI for this patient to assess what was contributing to this lack of functional recovery. Despite this outlier, there was an overall significant increase in FIM between admission and discharge in this patient group ($p = 0.0017$). We also observed a non-significant relationship regarding the correlation of FIM scores and length of stay

in rehab. There is a stronger negative correlation for FIM scores at the time of discharge and their total length of stay at the rehab facility (R^2 = 0.62), compared to their FIM scores at the time of admission and how long they stayed in rehab (R^2 = 0.29). Regarding the relationship of injury severity to FIM scores, there is a significant difference between ASIA A and ASIA D severities of SCI both at the time of admission and discharge (p = .047), and both groups show equally significant recovery in FIM over time (p < .0001). However, GCS severities for SCI do not show a significant trend to FIM scores, for either admission (R^2 = 0.13) or discharge (R^2 = 0.003).

SCI but not TBI severity significantly impacts length of stay in rehab. SCI and TBI severities were assessed with the ASIA and GCS measures, respectively, in comparison to length of stay in rehab to determine which type of injury is a stronger contributor to the time it takes for patients to be discharged. We found a significant increase in the length of stay for ASIA A subjects (71.7 ± 9.3 days) compared to ASIA D subjects (29.0 ±12.2 days, p = 0.02), however there was not a significant difference in length of stay between the different GCS severities either as groups (p = 0.52;, or as a correlation of GCS scores to length of stay (R^2 = 0.05). This is not explained by a higher incidence of severe TBI and SCI occurring together, since there was no significant difference in GCS scores between ASIA A and ASIA D groups (p = .32), and there does not appear to be a significant correlation between the neurological level of SCI and TBI severity (R^2 = 0.26). However, there is a large difference in the distribution of age at time of injury for males versus females. Even though most of the subjects in this cohort were males (71%), as a group they sustained their injuries over many age ranges (39 ± 17 years), whereas females only sustained their injuries at a very young age (19 ± 1.7 years).

The incidence of comorbid conditions in TBI/SCI patients was assessed. ICD-9 codes referenced in the medical records were mined and categorized for their respective conditions (see figure below) . Each condition was quantified for how many patients were diagnosed. The most prominent comorbid condition in these patients was neurogenic bowel (n=10) and bladder (n=9). This was also accompanied by a large number of patients also diagnosed with urinary tract infections (UTI) (n=9), consistent with autonomic dysfunction commonly seen in SCI patients [26, 27]. About half of the

patients also developed complications with swallowing (n=7), pressures sores (n=6), and hypertension (n=5), with additional complications associated with infections and neurological complications.



Medications suggest increased depression and infections, decreased pain, and continual constipation during length of stay. Medications referenced in the medical records were quantified for each patient at the time of admission and discharge to determine how many patients were being treated for various comorbid conditions, and how that changed during their length of stay. Most of the patients were treated for constipation at the time of admission (n=10), consistent with the number of comorbid cases of neurogenic bowel (see figure above), and all but 1 patient continued to suffer from this condition by the time of discharge. Almost half of the patients were being treated for depression by the time of discharge (n=6), and even fewer patients being treated for acid reflux, pain, and muscle spasms. Fortunately, most of the patients being treated for pain at the time of admission (n=8) no longer needed to take pain medication at discharge (n=1), and a few patients also no longer needed to be treated for other ailments, including anxiety, ulcers, infections, and other complications that presented at the time of admission.

Word frequency analysis of medical records highlight important information during recovery. We leveraged the admission and discharge notes to determine if additional useful information regarding these patients could be harnessed from word frequency analysis see figure below. There were more total words mined from the admission notes (19,552) than the discharge notes (12,484), and each word cloud is a representation of the percentage each word represents compared to the total words for each group. The four most relevant and frequent words seen in both the admission and discharge word clouds were fracture (1.26% and 1.23%), injury (0.95% and 0.91%), rehabilitation (0.58% and 0.74%) and pain (0.50% and 0.68%), respectively. We also assessed the relative

change these top occurring words were being mentioned between admission and discharge to highlight important changes in the state of the patients that may not be obvious from other aspects of the database. The percentages for each discharge word were subtracted

from the percentage for the same admission word and a new word frequency cloud was generated from the changes, and color coded to reflect increases (green) and decreases (red). Of these top four words, the mention of rehabilitation (+0.167%) and pain (+0.180%) both increased by the time of discharge. Additional words that increased in frequency that were noticed were the mention of improve (+0.118%), treatment (+0.115%), neurogenic (+0.101%) and hematoma (+0.118%). The most notable decreases in frequency over time were unable (-0.236%), cognition (-0.119%), impaired (-0.096%) and therapy (-0.144%). It was also noted that there was an increase in the mention of admission (+0.218%), and a decrease in the mention of discharge (-0.316%), which seems counter-intuitive at first. However, there may have been discussion about plans for discharge when the patient was admitted, and likewise the discharge notes may frequently reference improvements or changes from when they were admitted, and may be disregarded in future analyses.

In summary, we have succeeded in building a clinical TBI and SCI patient database from the Santa Clara Valley Medical Center models system database that details the acute as well as the chronic stage of recovery. We are currently preparing these data for publication (Guandique et al., 2013 in preparation).

3a2. SCI Service and the Polytrauma Center at VAPAHCS

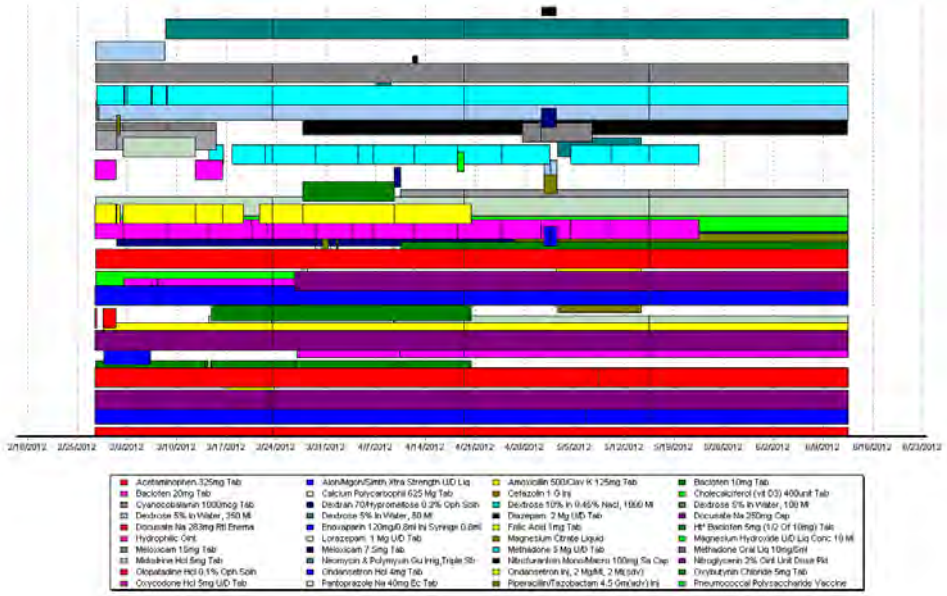
The strategy used with the Santa Clara Valley records was initially used as a model for extracting data on patients with TBI and SCI in the VA Palo Alto Health Care System, but the considerable differences between these two health care systems necessitated different approaches. The Polytrauma Service at VAPAHCS was founded in 2005, unlike the Model TBI System which has been in operation since 1989. As a result, a search of patients admitted for TBI rehabilitation only identified three patients who also had SCI. The records of patients recently admitted to the VA SCI Service for rehabilitation after acute SCI were therefore searched to identify those with TBI. The full text of all notes on these patients were searched for phrases such as “TBI” and “GCS,” and the context of these phrases was examined to determine the way they were used (for example, excluding patients in which the notes recorded that “TBI was ruled out”).

This search strategy showed that of an initial cohort of the 45 patients

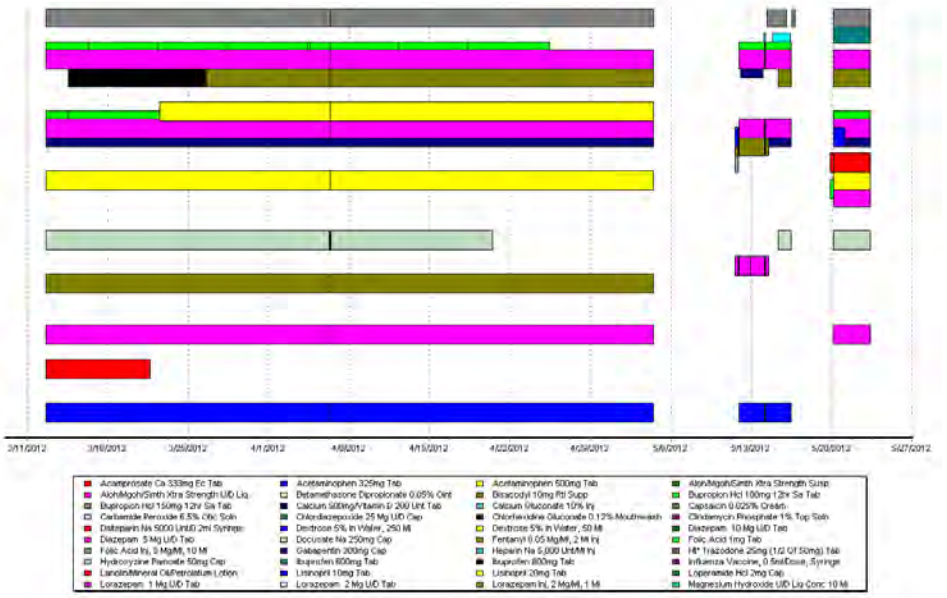
most recently admitted to the SCI Service for rehabilitation after acute SCI, ten (22%) had a TBI. As with SCVMC, we found that ICD-9 codes did not provide a reliable search strategy for identifying SCI and TBI patients.

To provide an initial overview of medications we produced a medication cloud that tracked duration of all medications prescribed during admission for acute rehabilitation. A medication cloud is shown for both a SCI patient and a TBI + SCI patient to provide a brief visualization of medications that were administered and their duration. Note the greater numbers of medications used in the Dual Diagnosis patient.

SCI+TBI patient at VA



SCI only patient at VA



A database of patients with spinal cord injuries or disorders currently served by the Spinal Cord Injury Service of the VA Palo Alto Health Care System was searched for patients who had been admitted for initial rehabilitation or subsequent care during the period October 1st 2010 to October 19th 2012. The electronic medical records of these patients in the VA Computerized Patient Record System were then searched for any reference to traumatic brain injury. The search strategy included examining the list of Active Problems and searching the text of all Notes electronically for any of the following text phrases: "TBI", "brain injury", "brain trauma", "head injury", "head trauma", "loss of consciousness", "LOC", "CVA" or "cognitive". When any of these words or phrases were found, their context was examined to determine whether the patient did indeed have a history of traumatic brain injury and its relationship in time to the spinal cord injury. Every note from the SCI Psychologists includes a section on cognitive functioning that was identified by this search; each of these notes was then read to determine whether there was cognitive impairment and whether it was attributable to the TBI or to other conditions. Other information was also extracted, such as age, gender, cause of SCI damage, and level and completeness of the spinal cord lesion.

701 patients with spinal cord injury or disorder were identified as having been admitted to the SCI Service either for initial rehabilitation or subsequent care, during a period of just over two years between October 1st 2010 and October 19th 2012. Of these, 675 were male and 26 were female, as is typical in the veteran population with SCI. 409 were identified as having sustained traumatic SCI and 292 as having non-traumatic SCI.

Traumatic and Non-traumatic SCI by Gender

SCI	**Male**	**Female**	**Total**	**Mean Age**	**Range**
Traumatic	400	9	409	60 ± 13	23 ÷ 93
Non-Traumatic	275	17	292	64 ± 13	24 ÷ 96
Total	**675**	**26**	**701**	**62 ± 13**	**23 ÷ 96**

Non-traumatic SCI by etiology

Etiology of SCI	Male	Female	Total
Arthritic	42		42
Infection or abscess	22	2	24
Herniated disc	6		6
Motor neuron disease (ALS)	32	1	33
MS	70	10	80
Multifocal motor neuropathy	1		1
Muscular Dystrophy	1		1
Myelopathy	28	1	29
Poliomyelitis	4		4
Syringomyelia	3		3
Tumor	26		26
Vascular change	22	1	23
Other non-traumatic	15	2	17
Unknown	3		3
Total	**275**	**17**	**292**

Of the 409 patients with traumatic SCI, 99, or 24.3%, had a TBI at the same time as the SCI and a further 17 had a TBI on a different occasion to the SCI. The level and completeness of the SCI in the 99 patients with a concurrent TBI is shown in the Table below.

Level and completeness of SCI in patients with concurrent TBI

	Tetraplegia	Paraplegia	Total
Complete	9	22	31
Incomplete	47	21	68
Total	**56**	**43**	**99**

Of the 99 patients with concurrent SCI and TBI, only 18 had TBI noted in their Active Problem list. The other patients were identified by electronically searching the text of notes. Most commonly, the TBI was mentioned in the text of notes by psychologists working in the SCI Service, and sometimes when reviewing a patient years after the injury, but not in the history recorded on admission.

The numbers of patients with concurrent SCI and TBI who were recorded as having cognitive impairment related to their TBI are shown in the Table below, sorted by etiology of SCI according to the

Common Data Elements classification. The most frequent cognitive impairments were short-term memory loss and slowed processing speed.

Cognitive impairments in vets with SCI+TBI by etiology of SCI

Etiology of SCI	**SCI with TBI**	**Cognitive Impairment**	**%**
Sports	12	6	50.0
Assault	2	1	50.0
Transport	58	30	51.7
Fall	24	17	70.8
Other traumatic	3	0	0.0
Total	**99**	**54**	**54.5**

The table below shows the etiology of SCI in all patients with traumatic SCI and in those with concurrent SCI and TBI. When the SCI was due to assault, concurrent TBI was rare. Of 47 cases of SCI due to assault, 39 were due to gunshot wounds and none of these 39 had concurrent TBI. The two cases with concurrent SCI and TBI due to assault were caused by shrapnel and a rocket propelled grenade respectively. If cases of assault are excluded, 97 out of 362 patients with SCI, or 26.8%, had suffered a concurrent TBI.

Traumatic SCI and TBI by etiology (females in parentheses)

Etiology	**Total**	**SCI with TBI**	**%**	**unrelated brain injury**
Sports	56	12	21.4	1
Assault	47	2	4.2	3
Transport	219 (7)	58 (3)	26.5	8
Fall	73 (1)	24 (1)	32.9	2
Other trauma	14 (1)	3 (1)	21.4	3
Total	**409 (9)**	**99 (5)**	**24.2**	**17**
Total (not including Assault)*	***362 (9)****	***97 (5)****	***26.8****	***14****

The table below shows when the concurrent injuries occurred in relation to military service. While 30 of the cases of SCI and TBI occurred during active military duty, the majority of these occurred during transport by road or air.

Concurrent SCI and TBI by relation to active military duty

Etiology of SCI	During active duty	After leaving military	Total
Sports	2	10	12
Assault	2	0	2
Transport	20	38	58
Fall	5	19	24
Other traumatic	1	2	3
Total	**30**	**69**	**99**

When patients are stratified according to the date of their traumatic SCI, it is notable that records of concurrent TBI have increased greatly over the last four decades. Since 2001 the prevalence of concurrent TBI in these patients with traumatic SCI has been recorded as 39.4%, and when patients whose SCI was caused by gunshot wound or shrapnel [assault?] are excluded, this figure rises to 42.7%. Possible reasons for this are discussed below.

Concurrent SCI and TBI by etiology and date of injury

	2012-2001			**2000-1991**			**1990-1981**			**1980-1952**		
Etiology of SCI	SCI	SCI + TBI	%	SCI	SCI + TBI	%	SCI	SCI + TBI	%	SCI	SCI + TBI	%
1.Sports	17	8	47.1	4	1	25.0	16	1	6.3	19	2	10.5
2.Assault	13	1	8.0	8	0	0	3	0	0	23	1	4.3
3.Transport	55	27	49.1	38	17	44.7	37	4	10.8	89	10	7.5
4.Fall	47	18	38.3	12	2	16.7	6	2	33.3	8	2	25.0
5.Other traumatic	5	0	0	2	0	25.0	2	1	50	5	2	20.0
Total	137	54	**39.4**	64	20	**31.3**	64	8	**12.5**	144	17	**11.8**
Total not including Assault	124	53	**42.7**	56	20	**35.7**	61	8	**13.1**	121	16	**13.2**

The prevalence of concurrent TBI in SCI patients in this retrospective study when averaged over six decades (24.2%) is similar to figures from the SCI Model Systems reported in 1995 (28.2%), but lower than figures from a prospective study in a single SCI Model System reported in 2008 (60%). SCI Model Systems tend to admit younger patients with a higher proportion of women, sometimes sooner after injury, However, the prevalence recorded in this study has increased significantly over this period.

There are several possible reasons for the increase in numbers of veterans with traumatic SCI recorded in this study as having a concurrent TBI, from less that 12% before 1980 to at least 40% since 2001.

a. military activity

In recent years, traumatic head injury has been described as the signature injury of military action in the Middle East, and it might be hypothesized that this has increased the number of SCI patients with TBI. However, patients whose SCI was caused by gunshot wound or shrapnel showed a much lower prevalence of TBI in this study, presumably because the missile struck either the spine or head but rarely both, and in recent conflicts the use of body armor appears to have greatly reduced the incidence of spinal cord injuries. It is well known that during active military duty many injuries are caused not in combat but by other forms of trauma such as motor vehicle accidents. In this study, while 30% of concurrent injuries occurred during active military duty, the majority of these occurred during transport by road or air. Only one was due to shrapnel and one was due to a rocket propelled grenade and none were due to gunshot. Only seven of 99 concurrent injuries occurred during combat: two occurred in motor vehicle accidents, three in flying accidents, only one was due to shrapnel and none to gunshot. It seems likely therefore that the contribution of military combat to increasing records of concurrent SCI and TBI is small.

b. improved documentation

The computerized Patient Record System was introduced in 1995. Patients injured before this time have their current medical records entered into this system but the records of their medical history before this time are heavily dependent on their memory, which can be impaired by head injury, and on being asked about the possibility of past head injury.

c. improved awareness

Awareness of head injury in military personnel has increased during the last two decades, and this has led to increased screening in the Department of Defense and in the Department of Veterans Affairs. Psychologists working in SCI units are usually aware of this, but other staff, including medical residents in training who may do much of the documentation, may be less aware of the possibility of head injury and less skilled in diagnosing it.

Traumatic SCI usually has obvious symptoms and signs and is

therefore relatively rarely missed, and major TBI is rarely missed. When both are present, management is usually assigned to either a SCI Unit or a TBI Unit, depending on the relative severity of the two injuries. Ideally the staff of such units would collaborate in the management of such patients. In practice, SCI Units and TBI Units may not be located in the same institution, and even when they are, they often have different cultures and collaboration may be limited. In practice each unit will concentrate on the injury it knows best, and the other injury may not receive state-of-the art attention.

Less severe TBI can be missed, particularly in patients with multiple and life-threatening injuries who may be in shock, undergoing emergency surgery, sedated, or on a ventilator. When they are stabilized, their management will depend somewhat on the service to which they are transferred, and on its awareness of the possibility of concurrent injuries.

Identification of TBI in records of patients with SCI in this study was inconsistent. It might be thought that this was because the head injury was mild in this series of patients, but Table 4 shows that over 50% of the veterans with concurrent TBI and SCI were identified as having cognitive impairment. This is similar to the percentage found in SCI patients treated in the Model SCI Systems of Care. While cognitive impairment can be due to causes other than TBI in these patients, it remains important to identify whether they have had a TBI.

In the case of patients with mild TBI, it might have been argued in the past that they did not suffer greatly from delayed or absent documentation of it, but there is now increased interest in the unknown long term effects of mild and repeated TBI on conditions such as Parkinson's disease and dementia. The fact that the VA follows patients with SCI for life offers an opportunity to study the relationship between these conditions.

The use of an electronic medical record in the VA has had many advantages, but it may be necessary to structure the collection and recording of some information in a more consistent way that could be implemented in a national system of care. Screening of SCI patients for TBI during their initial rehabilitation would help to avoid missing the diagnosis of TBI. If TBI resolves there is no way to identify it subsequently other than history from the patient, collaterals and prior medical reports. Screening will need to be done after patients are stabilized on medications for pain and spasticity since they are known to affect cognitive functioning until patients accommodate to them.

Patients will also need to be clear of delirium from surgical anesthesia, UTIs and other SCI complications. In many cases it will be impossible to distinguish TBI from depression, PTSD and/or anxiety, so diagnosis will be delayed until psychiatric symptoms are adequately treated. These are some of many reasons for providing adequate time for rehabilitation rather than discharging patients as soon as they can survive. Fortunately adequate admission time is standard practice in VA.

The PrOMOTE research project currently being carried out in the VA to study the effect of a more comprehensive approach to vocational rehabilitation is using detailed interviews of veterans in which they are asked about any prior history of head trauma. Of the first 100 SCI patients in the VA Palo Alto SCI Service to undergo these interviews, 70% reported having had a head injury, although not necessarily concurrently with their SCI. (Elspas - personal communication). It is possible therefore that the prevalence of 40-45% reported in this paper is still an underestimate, so there may still be a significant number of veterans in whom TBI has not been diagnosed.

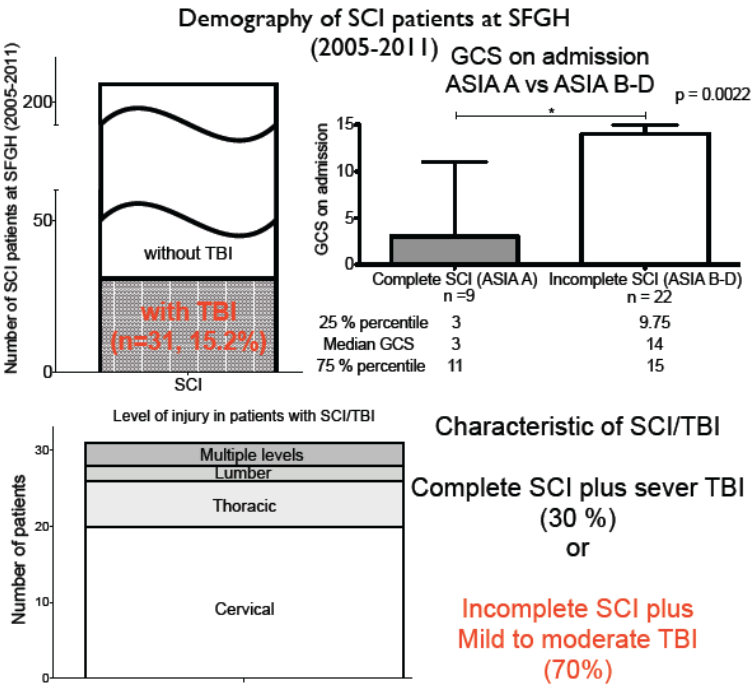
The causes of SCI in veterans with concurrent TBI resemble the causes seen in the civilian population. It would be of interest to determine comparable figures for the civilian population, particularly as electronic medical records are being adopted. This could best be done within the SCI Model Systems, even though only a minority of US civilians with SCI receive their care in this system and it may have a higher proportion of patients with severe spinal cord injuries than in the population treated outside the SCI Model Systems.

Conclusions

1. Documentation of TBI in this population of veterans with traumatic SCI was inconsistent: in patients with both SCI and TBI, the TBI identified by searching the Notes was only recorded among the Active Problems list in the electronic medical record 18% of the time, and was often absent from admission histories and discharge summaries.
2. Records of TBI in veterans with traumatic SCI in this study have increased from less than 12% before 1980 to over 40% since 2001. This may reflect improved documentation and increased awareness, but there may be further cases that are still not being identified. Extrapolation of these figures nationally suggests that there may be a substantial number of veterans whose TBI has not been documented.
3. Improved screening and documentation would help to identify all

SCI veterans with TBI and allow appropriate management and long term follow up.

3a3. Patients at UCSF/SFGH. Under the supervision of Dr. Manley, Dr. Tomoo Inoue examined the demographics and clinical picture for 203 SCI patients admitted to SFGH from 2005-2012. Of these, 31 were charted as having concurrent TBI. The ASIA grades were frequently not noted in the charts, and Dr. Inoue reviewed the available information and assigned a grade. In this data pool, approximately 15% had co-occurring TBI. These data were reported, in part, in an abstract presented at the annual Society for Neuroscience meeting in New Orleans (October, 2012)(Inoue et al, 2012). Patients with complete SCI were more likely to have a lower ASIA score than those with incomplete SCI. The level of injury was predominantly cervical, although a little over a third had thoracic, lumbar or multiple level injuries. Since patients treated at the acute neurotrauma center at SFGH are discharged to rehabilitation centers (including the SCVMC and, rarely, the VAPAHCS, rehabilitation measures are not easily available for this cohort, although we have excellent early data for them. One of the goals is to provide better early care data from the VA and SCVMC cohorts, and better long-term outcome data for the SFGH cohorts.



3b. Comparison of SCVMC, VAPAHCS, and SFGH/UCSF

A patient's rehabilitation outcome is greatly influenced by the amount of time that passes before they are admitted for rehabilitation. We determined the number of days between the date of injury and the date of admission into rehabilitation, and also the length of stay in

rehabilitation, and compared these between SCVMC and the VAPAHCS hospitals. The analysis is shown below:

Days till Admission to Rehabilitation		
Location	Mean	St Dev
VA (N = 10)	51.5	31.3
SCVMC (N = 14)	29.6	24.4

*No statistically significant difference (T-test)

Length of Stay in Rehabilitation		
Location	Mean	St Dev
VA (N = 10)	96.5	51.4
SCVMC (N = 14)	55.9	39.7

*Statistically significant difference (T-test, p <.05)

Note that the length of stay in rehabilitation is shorter in SCVMC, a civilian hospital, than VAPAHCS, a VA hospital.

The medications provided to Dual Diagnosis patients were compared between these two hospitals. The most common medications prescribed for these patients on admission to rehabilitation at each hospital are shown below:

Admission Medications			
VA	Frequency	SCVMC	Frequency
DOCUSATE	9	DOCUSATE	10
ACETAMINOPHEN	9	ACETAMINOPHEN	7
SENNA	9	SENNA	4
BISACODYL	7	BISACODYL	5
OMEPRAZOLE	7	OMEPRAZOLE	1
ALBUTEROL	6	ALBUTEROL	2
LIDOCAINE	5	LIDOCAINE	1
ASCORBIC ACID	4	ASCORBIC ACID	2
HYDROCODONE/ACETAMINOPHEN	4	HYDROCODONE/ACETAMINOPHEN	2
ONDANSETRON	4	ONDASENTRON	1
ENOXAPARIN	3	ENOXAPARIN	3
GABAPENTIN	3	GABAPENTIN	2
DOXYCYCLINE	3	DOXYCYCLINE	1
LISINOPRIL	3	LISINOPRIL	1
CHLORHEXIDINE	2	CHLORHEXIDINE	2
METOPROLOL	2	METOPROLOL	2
DEXTROSE	2	DEXTROSE	1
MICONAZOLE	2	MICONAZOLE	1
HEPARIN	1	HEPARIN	3
FENTANYL	1	FENTANYL	2
BACLOFEN	1	BACLOFEN	1
FERROUS SULFATE	1	FERROUS SULFATE	1
QUETIAPINE	1	QUETIAPINE	1

Note that the most common medications were those prescribed for management of the neurogenic bowel (e.g docusate, senna, bisacodyl), together with mild analgesics, antacids and bronchodilators.

We compared this with the medications prescribed at the time of discharge from rehabilitation. The most common medications prescribed on discharge are shown below:

Discharge Medications			
VA	Frequency	SCVMC	Frequency
ACETAMINOPHEN	10	ACETAMINOPHEN	7
DOCUSATE	8	DOCUSATE	8
OMEPRAZOLE	8	OMEPRAZOLE	1
SENNA	7	SENNA	4
LIDOCAINE	5	LIDOCAINE	1
ONDANSETRON	5	ONDASETRON	1
GABAPENTIN	4	GABAPENTIN	2
ASCORBIC ACID	4	ASCORBIC ACID	1
BISACODYL	4	BISACODYL	1
TRAZODONE	3	TRAZADONE	5
BACLOFEN	3	BACLOFEN	3
OXYCODONE	3	OXYCODONE	2
LACTOBACILLUS	3	LACTOBACILLUS	1
MICONAZOLE	3	MICONAZOLE	1
FERROUS GLUCONATE	2	FERROUS GLUCONATE	1
SIMVASTATIN	2	SIMVASTATIN	1
METOPROLOL	1	METOPROLOL	1

Note that some bowel medications (docusate and senna) are still among the most commonly prescribed, together with mild analgesics and antacids, but albuterol and low molecular weight heparin have been discontinued and the use of baclofen has increased. The most common medications with potential effects on the central nervous system at the time of admission are shown below.

Medications of Interest
HALDOPERIDOL, OLANZAPINE, RISPERDONE
IBUPROFEN, CELCOXIB, ASPRIN
LEVETIRACETAM, VALPROIC ACID
MODAFINIL, AMANTADINE
CITALOPRAM, BUPROPION
METOCLOPRAMIDE, DROPERIDOL
BACLOFEN
GABAPENTIN
LORAZEPAM
METHADONE
EPOETIN ALPHA
METFORMIN
DOCYCLINE

In the third year of the grant, pilot testing with some of these medications in the combined injury animal model was undertaken. We assessed topiramate as our first drug target. In addition, we plan to expand the clinical database to allow more detailed comparison of the SCVMC database with the VAPAHCS database so that both can be mined for hypotheses and refined through our community of practice and research.

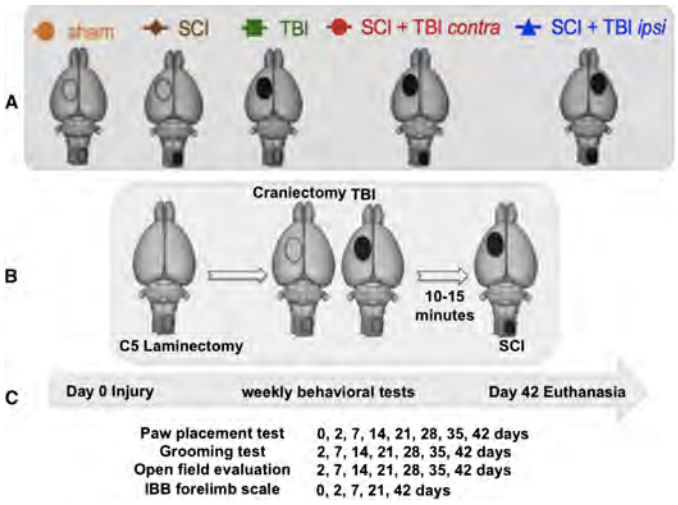
Specific Aim 2: Develop baseline incomplete SCI plus mild-complicated and moderate TBI rat protocols and outcomes

PI: Michael S. Beattie, PhD

Site: UCSF

Task 1: Factorial combination studies on SCI+TBI

An experimental animal model for combined SCI and TBI was developed to help drive mechanistic studies of dual diagnosis. A manuscript describing this model was published in Experimental Neurology (248:136-147, 2013). For these studies, rats received a unilateral SCI (75 kdyn) at C5 vertebral level, a unilateral TBI (2.0 mm depth, 4.0 m/s velocity impact on the forelimb sensori-motor cortex), or both SCI + TBI. TBI was placed either contralateral or ipsilateral to the SCI. Figure 1 above shows the experimental design.



Behavioral recovery was examined using a variety of outcome measures including paw placement in a cylinder (forebrain guided exploration), grooming, open field locomotion, and the IBB cereal eating test (object manipulation). Over 6 weeks, in the paw placement test, SCI + *contralateral* TBI produced a profound deficit that failed to recover, but SCI + *ipsilateral* TBI dramatically enhanced use of the paw on the SCI side. In the grooming test, SCI + *contralateral* TBI produced worse recovery than either lesion alone even though *contralateral* TBI alone produced no observable deficit. In the IBB forelimb test, SCI + *contralateral* TBI revealed a severe deficit that recovered in 3 weeks. For open field locomotion, SCI alone or in combination with TBI resulted in an initial deficit that recovered in 2 weeks.

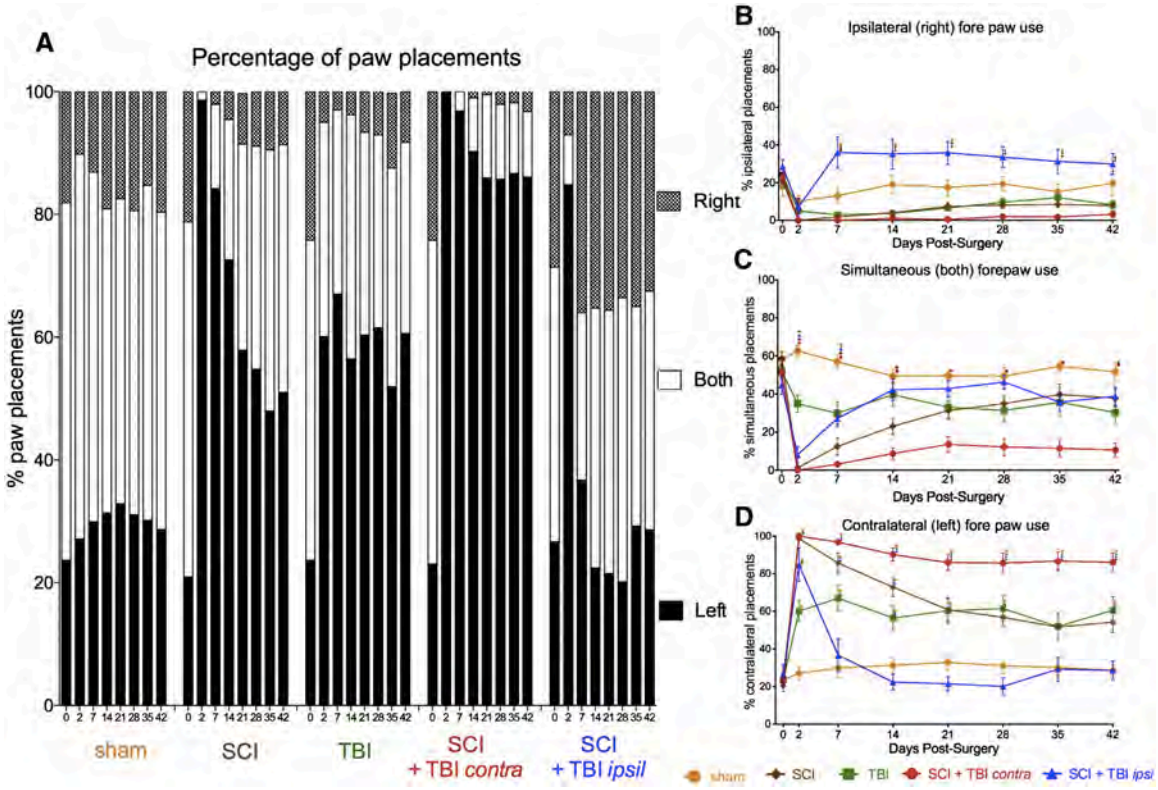


Fig. 2 (above). Paw placements in the cylinder. (A) The percentage of total paw placements (left, right, or both) is shown for each group. (B) The proportion of total paw placements made by the ipsilateral (right) paw is shown. (C) The proportion of total paw placements made simultaneously by both paws is shown. (D) The proportion of total paw placements made by the contralateral (left) paw is shown. (A-D) The performance of sham rats did not significantly differ from pre-injury at any time post-operatively. SCI alone rats showed a profound deficit that recovered to the level of TBI alone rats over 42 days weeks. TBI produced a stable deficit in pawplacement that did not recover over 6 weeks. SCI + contralateral TBI produced a profound deficit that failed to recover over 42 days, showing an almost complete preference for the limb contralateral to the SCI. SCI + ipsilateral TBI rats initially did not use the right forepaw (2 days after

the injury) but they then significantly increased right limb use (i.e. ipsilateral to the SCI). (B) SCI + ipsilateral TBI significantly increased ipsilateral forepaw use compared to SCI + contralateral TBI rats 7 days after surgery. (C) SCI + contralateral TBI significantly reduced simultaneous forepawuse compared to sham. (D) SCI + contralateral TBI significantly enhanced contralateral forepaw use compared to both sham and SCI + ipsilateral TBI rats 7 days after surgery. SCI: spinal cord injury, TBI: traumatic brain injury, open circle: significant difference compared to sham group,♢:significant difference compared to SCI group, open box: significant difference compared to TBI group, #:significant difference compared to SCI + ipsilateral TBI group.

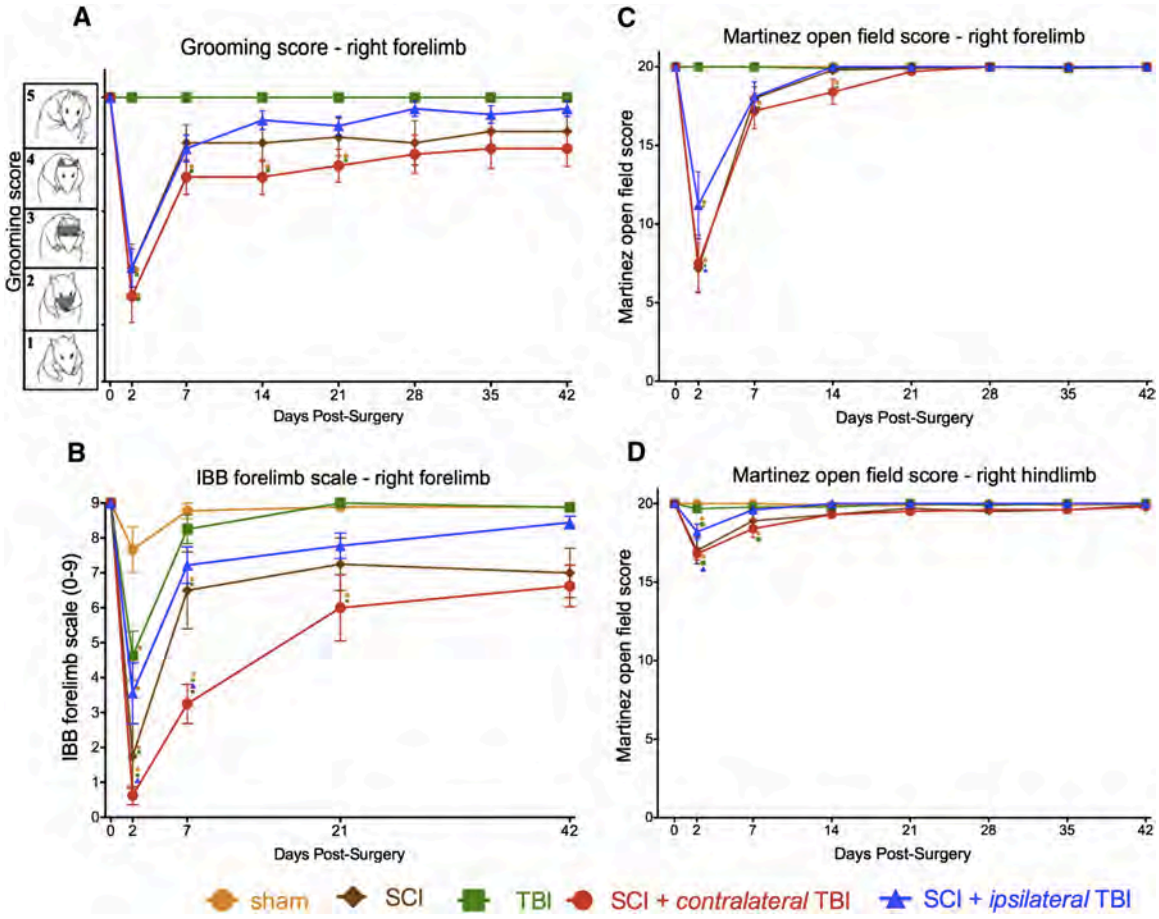


Fig. 3 (above). Grooming, paw use (IBB) and open field locomotor performance over time after injury. (A) Both sham lesions and TBI alone did not affect grooming, but rats with SCI (SCI alone and combined SCI and TBI) produced deficits in grooming. Two days after injury, the majority of animals with SCI were only able to touch the bottom of the snout (score of 1), but they remarkably improved from 2 days to 7 days post-injury. Twenty-one days after the injury, only SCI + contralateral TBI rats demonstrated significantly lower grooming scores than sham and TBI only groups (score of 4). Forty-two days after injury, no statistically significant differences between groups were evident. (B) Irvine

Beatties Bresnahan (IBB) forelimb scale. (Irvine et al., 2010). Before the injury, the rats could eat any type of cereal without any deficit in forelimb function (score of 9); but 2 days after the injury, rats with both SCI and TBI showed a significant decrease in ipsilateral (right) forepaw function. Seven days after the injury, only the SCI and SCI + contralateral TBI rats showed deficits in forelimb function. (C) Open field forelimb locomotor test using the Martinez score (Martinez et al., 2009). Neither sham nor TBI alone affected forelimb locomotor scores, but rats with SCI (SCI alone and combined SCI and TBI) exhibited deficits in locomotor function. Rats with SCI showed severe impairments of forelimb movements and postural abilities at 2 days post-injury but rapidly recovered within the first 7 days. SCI + contralateral TBI rats recovered more slowly than SCI alone and SCI + ipsilateral TBI rats, but finally reached a similar level of motor skills. (D) Openfield hindlimb locomotor test. During the first 7 days after the injury, rats with SCI showed hindlimb deficits, which were mainly characterized by poor stepping. SCI: spinal cord injury, TBI: traumatic brain injury, open circle: significant difference compared to sham group, open square: significant difference compared to TBI group, filled triangle: significant difference compared to SCI + ipsilateral TBI group.

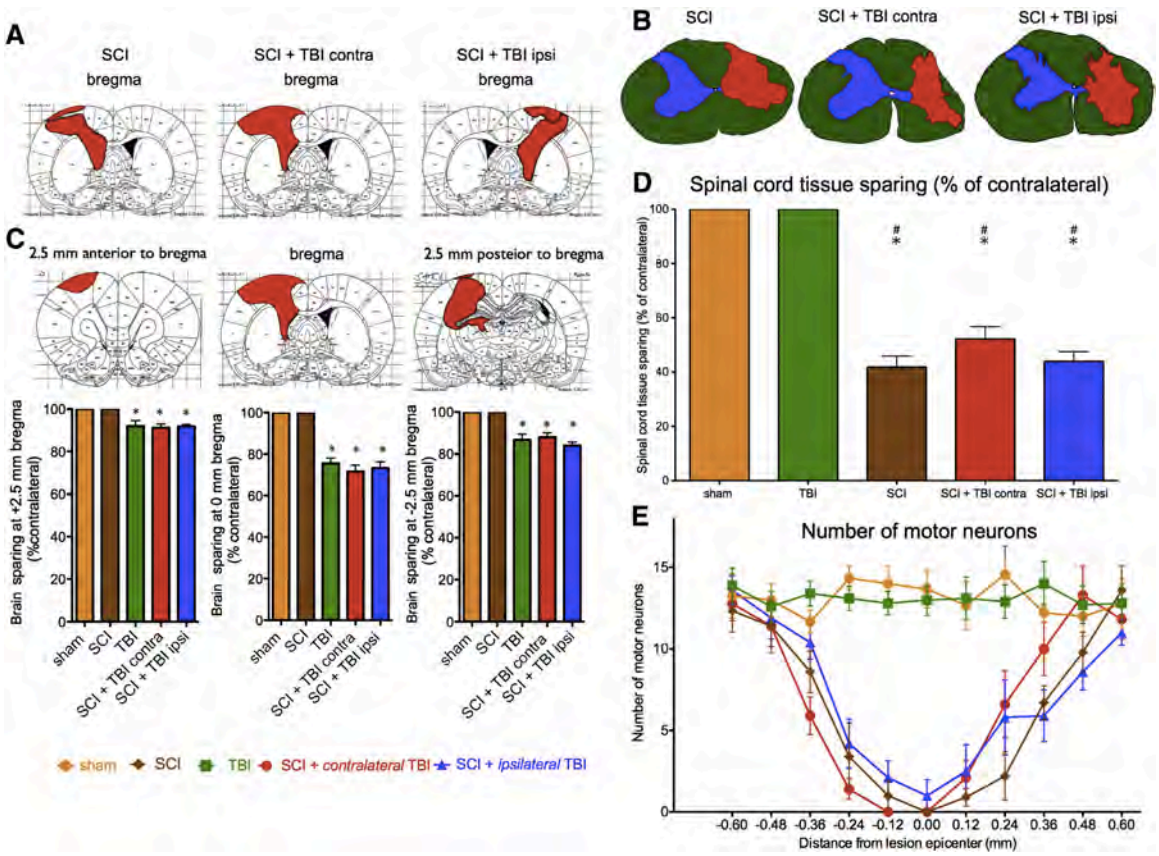


Fig. 4. Both brain (A) and spinal cord (B) lesions were not significantly different in size or location between groups. The median lesions are shown for each group (A,B), and for each location. (C). The median tissue sparing at 2.5 mm anterior to

bregma, at bregma, and 2.5 mm posterior to bregma is shown below (*p b 0.05 vs. Sham or SCI alone). (D) Spinal cord sparing at the epicenter of the lesion is shown (#p b 0.05 vs. sham; *p b 0.05 vs. TBI). (E) Motor neuron counts throughout the extent of the lesion show no significant differences between injury groups. SCI: spinal cord injury, TBI: traumatic brain injury.

Thus, TBI and SCI affected forelimb function differently depending upon the test, reflecting different neural substrates underlying, for example, exploratory paw placement and stereotyped grooming. Concurrent SCI and TBI had radically different effects on outcomes and recovery, depending upon laterality of the two lesions. Recovery of function after cervical SCI was retarded by the addition of a moderate TBI in the contralateral hemisphere, but recovery was markedly enhanced by an ipsilateral TBI. These findings emphasize the complexity of recovery from combined CNS injuries, and the possible role of plasticity and laterality in rehabilitation, and provide a start towards a useful preclinical model for evaluating effective therapies for combine SCI and TBI.

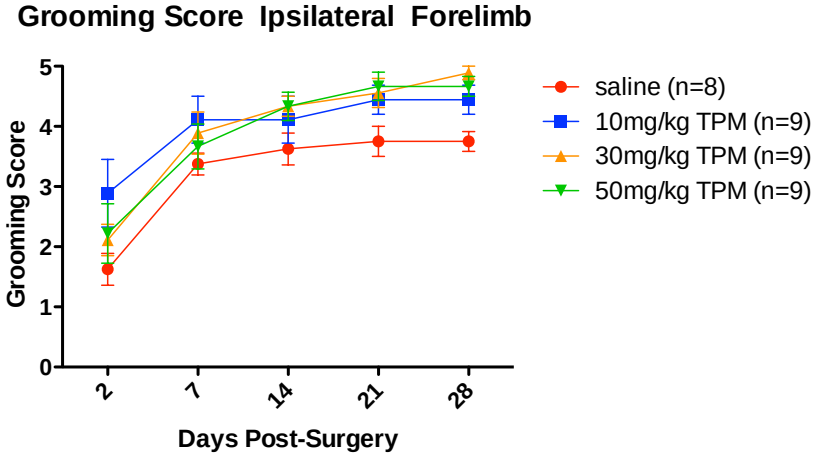
Milestone: This paper (Inoue et al., 2013) is included in the appendix.

Specific Aim 3: Test clinic-driven hypotheses for improving outcomes in the dual diagnosis animal model

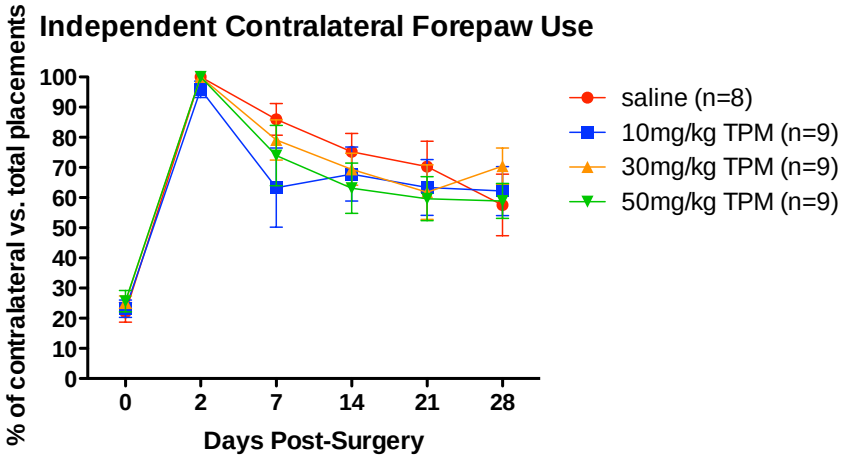
PI: Michael S. Beattie, PhD

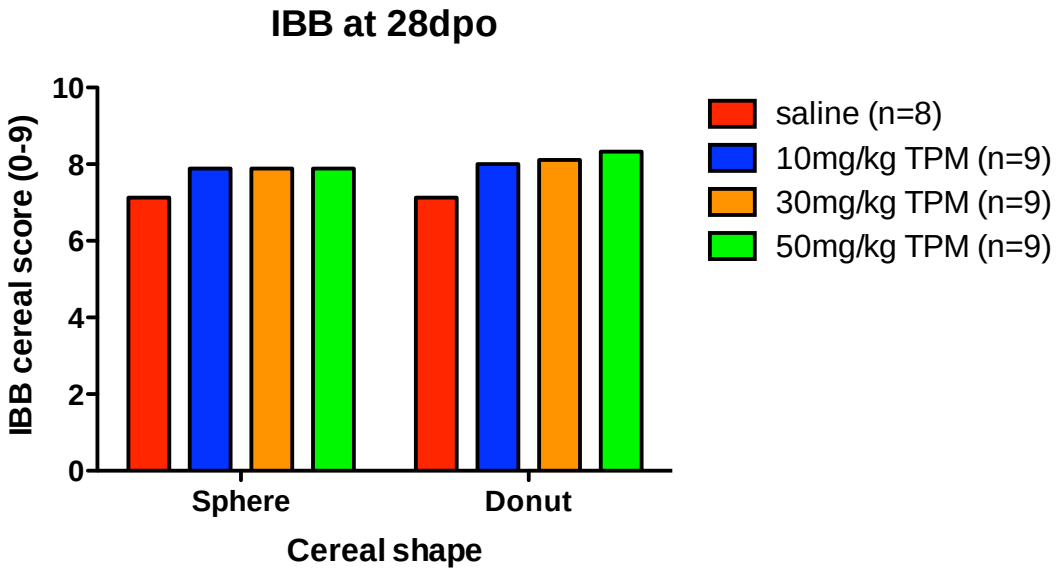
Site: UCSF

Tasks 1-3. Based on the outcomes of the model development studies, we decided to combine Tasks 1-3 by selecting a treatment that has multiple targets. The drug, topiramate, has a variety of properties including anti-epileptic, analgesic and neuroprotective qualities (Angehagen et al, 2003) and is a currently FDA approved agent which makes it potentially available for rapid clinical application. It has been shown to reduce allodynia and hyperalgesia in a pain model of chronic nerve constriction (Benoliel et al, 2006); reduce lesion size in stroke models and reduce consequent behavioral deficits as well (refs). Thus, topriamate seemed a good drug to test for efficacy in both the TBI and SCI contexts. To determine whether this drug has efficacy in a model of SCI, and to identify an effective dose, we first tested topiramate in a dose-response study using the spinal cord injury model alone. We found that topiramate at all doses improved forepaw use for



grooming behavior (see graph above), as well as spontaneous forepaw use in the cylinder (see graph below; better performance is a lower score in this schema), and for food manipulation testing using the IBB cereal eating test (data shown below), all tests that were differentially sensitive in the combined injury model. Topiramate also appeared to reduce tissue damage suggesting that after SCI, it is neuroprotective. (These data will be presented at the Society for Neuroscience Annual Meeting (Nov. 3, 2013; Beattie et al., 2013).



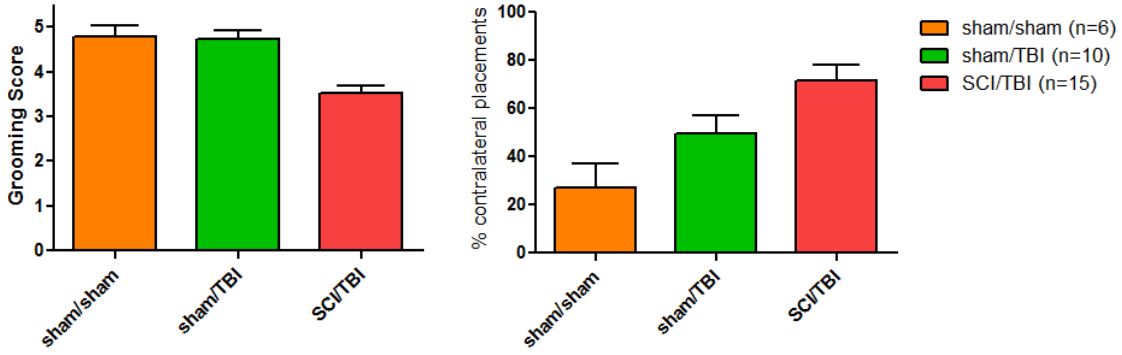


We then chose to examine the 50 mg/kg dosing of topiramate (TPM) in a second phase of the experiment, as this dose is frequently used clinically for seizure control. The following six groups were included:

1) sham SCI, sham TBI, TPM treatment (n=3)
2) sham SCI, sham TBI, Saline (n=3)
3) sham SCI, TBI, TPM treatment (n=5)
4) sham SCI, TBI, Saline (n=5)
5) SCI, TBI contralateral to the SCI, TPM (n=8)
6) SCI, TBI contralateral to the SCI, Saline (n=7)

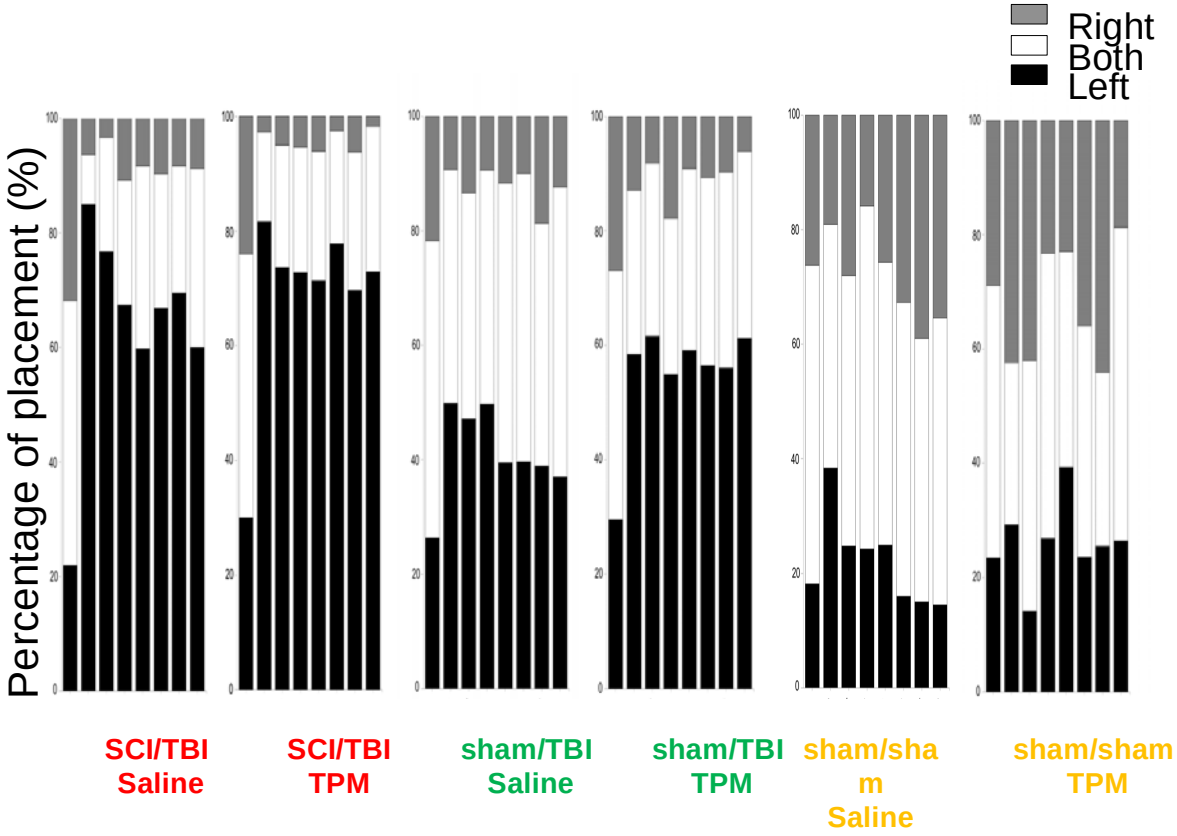
The first two groups are treatment and surgical controls; the second two groups have TBI lesions, one with and one without topiramate a treatment, and the 5th and 6th groups have a combined injury (i.e. both a SCI and TBI contralateral to the SCI), one with and one without topiramate treatment.

First, we replicated the finding from Inoue, et al. (2013) on the primary outcome measures (grooming, paw placement, and cereal eating), showing a differential effect of lesion condition (TBI alone, and SCI+TBI contra) on grooming and paw placement (both main effects $p<0.001$).



The treatment with topiramate, however, did not affect recovery of grooming across groups, but grooming was little affected by TBI. Paw placement showed an interesting and unexpected response to the treatment. The illustration below shows paw use for each paw independently (right or left) and together (both) over the 6 week observation period. The right paw is ipsilateral to the spinal cord injury and contralateral to the TBI. Comparing the sham groups (far right 2 groups), one can see that the right and left paw are used independently about equally and both are used about half the time. Introducing a TBI (middle two groups) causes the right paw (contralateral to the lesion) to be used significantly less and the left paw and both paws more. The topiramate treatment exacerbated this effect. The SCI plus TBI showed the greatest effect on the right paw use and the topiramate worsened this slightly. TPM produced a non-significant ($p = .057$) trend for worse recovery pooled across the TBI and TBI+SCI

groups on paw placement. The histological analysis for this study is underway. We think that this is a potentially interesting example of what we hypothesized in



the grant--a drug therapy that has a beneficial effect and in SCI, but may impair recovery in SCI+TBI.

This study is being prepared for publication (Morioka et al., 2013).

Specific Aim 4: Combine information from clinical practice queries and animal model results to plan for dual diagnosis guidelines
PIs: G. Creasey, MD, S. McKenna, MD, G. Manley, MD, PhD, M. Beattie, PhD
Site: VAPA, SCVMC and UCSF
Tasks 1 - 3. Begin to gain consensus on needed changes in current practice, begin the process for establishing new guidelines for dual diagnosis treatment and continue community input into hypotheses to be tested in the animal model. The community of practice and research has been established, and while this last specific aim may have been somewhat over-ambitious for this 3 year award,

the group continues to meet for this purpose. We will continue to interact for the next three years under the support of the DoD on another award that has similar goals of matching animal models with clinical reality.

KEY RESEARCH ACCOMPLISHMENTS.

- Established a community of practice and research, for the promotion of clinical and basic scientific interaction around the problem of combined brain and spinal cord injury.
- Developed clinical database search strategies and gathered dual diagnosis data showing that combined injuries are frequent and not typically diagnosed as such, resulted in different treatments (e.g. more medications), and had different outcomes than each injury alone.
- Developed a rat model of combined spinal cord injury plus mild-complicated and moderate traumatic brain injury, and used this model for an initial evaluation of topiramate.

REPORTABLE OUTCOMES

Meetings and Papers

National Neurotrauma Society Annual Meeting, Fort Lauderdale, Fl 2011 Combined traumatic brain injury and cervical spinal cord injury in the rat: Additive and dissociated effects on neurological outcomes. Tomoo Inoue, Amity Lin, Xiao Kui Ma, Jinghua Yao, Xiaoming Yao, Yvette Nout, Stephen McKenna, Graham Creasey, Geoffrey T. Manley, Adam R. Ferguson, Jacqueline C. Bresnahan, Michael S. Beattie.

Santa Clara Valley Brain Injury Conference, February 24-26, 2011.
"Dual Diagnosis with Brain and Spinal Cord Injury: An Interactive Assessment."

VAPAHCS TBI Research Forum, March 16, 2012
"Combined traumatic brain injury and cervical spinal cord injury in the rat: additive and dissociated effects on neurological outcomes."
Inoue T, Lin A, Ma X, Nout Y, McKenna S, Creasey G, Manley G, Ferguson R, Bresnahan J, Beattie M.

International Spinal Cord Society Meeting, London, England July 2012. Effects of combined unilateral cervical spinal cord injury (SCI) and traumatic

brain injury (TBI) in the rat. J.C. Bresnahan, T. Inoue, G. Creasey, S. McKenna, A. Ferguson, G. Manley, M. Beattie

Society for Neuroscience Annual Meeting, New Orleans LA, October 12-17, 2012. "Combined brain and spinal cord injury: Clinical picture and an animal model." Inoue T, Lin A, Ferguson A, Creasey G, McKenna S, Manley G, Bresnahan J, Beattie M.

VAPAHCS TBI Research Forum, March 15, 2013
Development of a database for combined brain and spinal cord injury. Guandique, C.F.[1], Nielson, J.L.[1], Arellano, C.A.[1], Kosarchuk, J.J.[2], Doan, R.[2], Inoue, T.[1], Wright, J.[2], Manley, G.T.[1], McKenna, S.L.[2], Creasey, G.H.[3], Bresnahan, J.C.[1], Beattie, M.S.[1], Ferguson, A.R.[1].

Society for Neuroscience Annual Meeting, Nov 2013. Big –data visualization for translational neurotrauma. Neilson JL, Inoue T, Paquette J, Lin A, Sacramento J, Liu AW Guandique CF, Irine KA, Gensel JC, Manley GT, Carlsson GE, Lum PY, Beattie MS, Bresnahan JC, Ferguson AR.

Society for Neuroscience Annual Meeting, Nov 2013. The AMPA receptor antagonist topiramate improves recovery of function following unilateral cervical contusion injury. Beattie, M.S., Lin, A., Huie, J.R., Ferguson, A.R., Bresnahan, J.C.

Inoue T, Lin A, Ma X, McKenna S, Creasey GH, Manley GT, Ferguson AR, Bresnahan JC, Beattie MS. Combined SCI and TBI: Recovery of forelimb function after unilateral cervical spinal cord injury (SCI) is retarded by contralateral traumatic brain injury (TBI), and ipsilateral TBI balances the effects of SCI on paw placement. Exp Neurol, 2013; 248:136-147.

Manuscripts in Preparation

Guandique CF, Nielson JL, Kosarchuk JJ, Wright j, Doan R, Inoue T, Liu AW, Ferguson AR, Manley GT, Bresnahan JC, Creasey GH, Beattie MS, McKenna SL (2013) Development of a Translational Database for Combined Traumatic Brain Injury and Spinal Cord Injury. Manuscript in preparation.

Creasey GH, et al. (2013) Prevalence of traumatic brain injury among US veterans with spinal cord injury. Manuscript in preparation.

CONCLUSION

This project has accomplished the majority of its tasks for this Translational Research Partnership. We have developed a community of practice and research for SCI and TBI in the San Francisco Bay Area of California and conducted focus groups to determine needs and attitudes of clinicians and others to these diagnoses and the potential for modeling the combined diagnosis in animals. We have queried several databases representing veterans and civilians with TBI and SCI and conducted a preliminary merge of clinical databases available for these diagnoses and developed a search strategy for determining the scope of the problem and the areas of priority for animal modeling. On this basis, a rodent model of combined SCI + TBI has been designed and created by the Principal Investigators at the Brain and Spinal Injury Center at UCSF, and has been used to compare the outcomes of SCI, TBI and combined SCI and TBI in this animal model. We have tested a treatment in this model and have found interesting interactions between the SCI and combined SCI+TBI models. An ongoing collaboration has been established between the Principal and Partnering Investigators to interpret the data being obtained, and to define improved outcome measures and treatment practice information based on both the new animal model of combined injury and the merged databases.

Appendix: Inoue et al., 2013

Experimental Neurology 248 (2013) 136–147

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Combined SCI and TBI: Recovery of forelimb function after unilateral cervical spinal cord injury (SCI) is retarded by contralateral traumatic brain injury (TBI), and ipsilateral TBI balances the effects of SCI on paw placement

Tomoo Inoue [a,b,1], Amity Lin [a,b,1], Xiaokui Ma [a,b,1], Stephen L. McKenna [c,2], Graham H. Creasey [d,3], Geoffrey T. Manley [a,b,1], Adam R. Ferguson [a,b,1], Jacqueline C. Bresnahan [a,b,1], Michael S. Beattie [a,b,*]

[a] Department of Neurological Surgery, University of California San Francisco, and San Francisco General Hospital, San Francisco, CA, USA
[b] Brain and Spinal Injury Center, University of California San Francisco, San Francisco, CA, USA
[c] Rehabilitation Trauma Center, Santa Clara Valley Medical Center, San Jose, CA, USA
[d] Spinal Cord Injury and Disorders Service, Department of Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA

ARTICLE INFO

Article history:
Received 19 December 2012
Revised 23 May 2013
Accepted 6 June 2013
Available online 13 June 2013

Keywords:
Behavioral analysis
Neuronal plasticity
Spinal cord injury
Traumatic brain injury
'Dual diagnosis'

ABSTRACT

A significant proportion (estimates range from 16 to 74%) of patients with spinal cord injury (SCI) have concomitant traumatic brain injury (TBI), and the combination often produces difficulties in planning and implementing rehabilitation strategies and drug therapies. For example, many of the drugs used to treat SCI may interfere with cognitive rehabilitation, and conversely drugs that are used to control seizures in TBI patients may undermine locomotor recovery after SCI. The current paper presents an experimental animal model for combined SCI and TBI to help drive mechanistic studies of dual diagnosis. Rats received a unilateral SCI (75 kdyn) at C5 vertebral level, a unilateral TBI (2.0 mm depth, 4.0 m/s velocity impact on the forelimb sensori-motor cortex), or both SCI + TBI. TBI was placed either contralateral or ipsilateral to the SCI. Behavioral recovery was examined using paw placement in a cylinder, grooming, open field locomotion, and the IBB cereal eating test. Over 6 weeks, in the paw placement test, SCI + *contralateral* TBI produced a profound deficit that failed to recover, but SCI + *ipsilateral* TBI increased the relative use of the paw on the SCI side. In the grooming test, SCI + *contralateral* TBI produced worse recovery than either lesion alone even though *contralateral* TBI alone produced no observable deficit. In the IBB forelimb test, SCI + *contralateral* TBI revealed a severe deficit that recovered in 3 weeks. For open field locomotion, SCI alone or in combination with TBI resulted in an initial deficit that recovered in 2 weeks. Thus, TBI and SCI affected forelimb function differently depending upon the test, reflecting different neural substrates underlying, for example, exploratory paw placement and stereotyped grooming. Concurrent SCI and TBI had significantly different effects on outcomes and recovery, depending upon laterality of the two lesions. Recovery of function after cervical SCI was retarded by the addition of a moderate TBI in the contralateral hemisphere in all tests, but forepaw placements were relatively increased by an ipsilateral TBI relative to SCI alone, perhaps due to the dual competing injuries influencing the use of both forelimbs. These findings emphasize the complexity of recovery from combined CNS injuries, and the possible role of plasticity and laterality in rehabilitation, and provide a start towards a useful preclinical model for evaluating effective therapies for combine SCI and TBI.

Abbreviations: ANOVA, analysis of variance; BBB, Basso,Beattie,Bresnahan Locomotor Rating Scale; CCI, controlled cortical impact; CT, computed tomography; HD, high definition; IBB, Irvine,Beatties,Bresnahan forelimb rating scale; IH, Infinite Horizons impactor; MRI, magnetic resonance imaging; PBS, phosphate buffered saline; SCI, spinal cord injury; SEM, standard error of the mean; TBI, traumatic brain injury.

* Corresponding author at: Brain and Spinal Injury Center, Department of Neurological Surgery, 1001 Potrero Ave., Bldg 1, Rm 101, University of California, San Francisco, CA 94110, USA. Fax: +1 415 206 3948.

E-mail addresses: inouet@neurosurg.ucsf.edu (T. Inoue), Amity.Lin@ucsf.edu (A. Lin), MaX@neurosurg.ucsf.edu (X. Ma), Stephen.McKenna@hhs.sccgov.org (S.L. McKenna), Graham.Creasey@va.gov (G.H. Creasey), manleyg@neurosurg.ucsf.edu (G.T. Manley), adam.ferguson@ucsf.edu (A.R. Ferguson), jacqueline.bresnahan@ucsf.edu (J.C. Bresnahan), michael.beattie@ucsf.edu (M.S. Beattie).

[1] University of California, San Francisco, Department of Neurological Surgery, and San Francisco General Hospital, 1001 Potrero Avenue, Building 1, Room 101, San Francisco, CA 94110. Fax: +1 415 206 3948.

[2] Rehabilitation Trauma Center, Santa Clara Valley Medical Center, 751 S Bascom Avenue, San Jose, CA.

[3] Spinal Cord Injury and Disorders Service at Department of Veterans Affairs Palo Alto Health Care System, 3801 Miranda Avenue, Palo Alto, CA.

0014-4886/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.expneurol.2013.06.006

Introduction

The coincidence of spinal cord injury (SCI) and traumatic brain injury (TBI) has long been acknowledged in trauma patients and such co-morbidity can present a significant problem for determining the best approaches to clinical management and rehabilitation (Arzaga et al., 2003; Bradbury et al., 2008; Cooper and Ackland, 2005; Davidoff et al., 1985a, 1985b, 1986, 1988; Hagen et al., 2010; Holly et al., 2002; Iida et al., 1999; Macciocchi et al., 2004, 2008; Michael et al., 1989; O'Malley and Ross, 1988; Povolny and Kaplan, 1993; Richards et al., 1991; Ricker and Regan, 1999; Soicher and Demetriades, 1991; Sommer and Witkiewicz, 2004; Stambrook et al., 1991; Tian et al., 2009; Watanabe et al., 1999; Wei et al., 2008). Published incidence rates range from 16% to 74% (Hagen et al., 2010); and, in a prospective study, 34% of co-occurring TBIs were mild and 26% were severe (Macciocchi et al., 2008). TBI complications in military personnel in Iraq are associated with a comorbid incidence of SCI (9.8%) (Bell et al., 2009). There is a need to identify factors that limit functional gains as well as a need to develop specific treatment strategies for patients with SCI and TBI, but few specific "dual diagnosis" standards of care are available at present (Arzaga et al., 2003; Ricker and Regan, 1999; Sommer and Witkiewicz, 2004). The complications associated with "dual-diagnosis" such as cognitive or behavioral dysfunction, are well known in the rehabilitation setting (Arzaga et al., 2003; Ricker and Regan, 1999; Sommer and Witkiewicz, 2004), but evidence-based approaches for treatment are lacking. In the clinical setting, mild or moderate TBI is sometimes overlooked in SCI patients because the paralysis is so clinically striking (Arzaga et al., 2003; Ricker and Regan, 1999; Sommer and Witkiewicz, 2004). In such SCI patients, TBI may first manifest as the inability to learn their rehabilitation protocols or to accomplish functional tasks expected at their level of injury (Arzaga et al., 2003). These patients may be labeled as having maladaptive psychological reactions or as being non-compliant (Sommer and Witkiewicz, 2004). The cognitive or behavioral disturbances consequent to the TBI need to be evaluated and treated as intensively as the SCI deficits. Without positive findings on computed tomography (CT) or magnetic resonance imaging (MRI), the screening of either mild or moderate TBI can be often excluded from further, more accurate diagnostic evaluation (Ricker and Regan, 1999). After the injury, the patient's communication is often compromised by sedation, intubation, or impaired consciousness, and mild or moderate TBI may be misdiagnosed as depression, denial, psychiatric disorders, or intensive care unit psychosis (Ricker and Regan, 1999).

Currently the clinical management of patients with both SCI and TBI is highly variable as in the rehabilitation phase, patients are often assigned to a rehabilitation unit specializing in either SCI or TBI depending on which injury appears more severe. Rehabilitation of one injury is not necessarily well integrated with rehabilitation of the other, and may be complicated by insufficient understanding of their interaction. Hence, there is a mounting need to advance our understanding of the mechanistic consequences of combined injury. This is especially true given the apparent importance of cortical plasticity in the recovery of function after partial SCI (Kokotilo et al., 2009; Nishimura and Isa, 2009, 2012). It could be expected that the addition of cortical and other forebrain injury to a SCI might retard recovery by reducing the capacity for cortical plasticity. On the other hand, some studies of unilateral cortical damage, which often occurs in the clinical setting, have suggested that such damage can actually enhance forelimb function by promoting plasticity in the contralateral cortex (Allred et al., 2010; Jones et al., 1996, 2009, 2012).

There is at present no preclinical model of combined SCI and TBI to enhance our mechanistic understanding of the interactions between these comorbidities. Thus, the biological and behavioral effects invoked by concurrent mild, mild-complicated, or moderate TBI in the outcome of SCI are largely unknown. This argues for: 1) capturing more information on concurrent SCI and TBI in clinical practice and in clinical trials, and 2) using this clinical information to guide the establishment of reliable and useful animal models of dual diagnosis. We have formed a translational partnership between basic scientists and clinicians to develop the first animal model of combined brain and spinal cord injury. In this first iteration of that model, we used unilateral contusion lesions of the cervical cord and the somatomotor cortical surface to examine these interactions. Rats were given controlled cortical contusion injury followed immediately by a unilateral cervical spinal cord contusion injury using a balanced experimental design (Fig. 1). We monitored forelimb behavioral recovery using standardized scales for 6 weeks, and terminal histopathology was characterized. The data revealed novel interacting features of TBI and SCI that emphasize the complexity of these interactions, including a dissociation of apparent retardation and enhancement of recovery from SCI depending upon the laterality of the TBI. This model can now be experimentally probed using clinically relevant therapeutics.

Materials and methods

For the purposes of the current paper the term *ipsilateral* refers to the side ipsilateral to the SCI (right side of the animal) and *contralateral* refers to the side contralateral to the SCI (the left side of the animal).

Animals

Female Long-Evans hooded rats (Simonsen Laboratories, Gilroy, CA, USA) with a mean age of 77 days (range; 75–80) and mean weight of 230 g (range: 198–257) were used in this study. Rats were housed individually in plastic cages, maintained on a 12 h light/dark cycle, and had access to food and water ad libitum. All animal experiments were approved by the Institutional Laboratory Animal Care and Use Committee of the University of California at San Francisco and were performed in compliance with NIH guidelines and recommendations. Surgical procedures were carried out aseptically under deep anesthesia induced and maintained by inhalation of isoflurane (IsoFlow, Abbott Laboratories, North Chicago, IL, USA; 2–3%), and anesthetic plane was monitored using withdrawal to foot pinch. Animals were administered cefazolin (Ancef, Novation, LCC, Irving, TX) 25 mg/kg, prior to surgery and for 3 days postoperatively. Lacrilube ophthalmic ointment (Allergan Pharmaceuticals, Irvine, CA, USA) was applied to the eyes prior to surgery and body temperature was monitored using a rectal thermal probe and maintained at 37.5 ± 0.5 °C using a heating pad.

Combined injury models

The following experimental groups (see Fig. 1) were compared: sham (craniectomy + laminectomy; n = 9), SCI + craniectomy (n = 10), TBI + laminectomy (n = 10), SCI + *contralateral* TBI (n = 10), SCI + *ipsilateral* TBI (n = 10). Group comparisons of both initial deficit and long term recovery were made. During surgery, a spinal laminectomy was made first, then a craniectomy, followed by a TBI for those groups with TBI, and then a SCI for those groups with SCI. Due to procedural limitations on transferring animals between the two injury devices, SCI was performed approximately 10+ minutes after TBI in the dual injury groups: SCI + *contralateral* TBI and SCI + *ipsilateral* TBI (Fig. 1B).

Traumatic brain injury (controlled cortical impact (CCI)) injury model

We used a well-validated controlled TBI device that has been described in detail elsewhere (Dennis et al., 2009; Igarashi et al., 2007; Lu et al., in press). Briefly, the rats were mounted in a Kopf stereotaxic frame under isoflurane anesthesia. A unilateral craniectomy (6.0 mm diameter) was produced in the skull between 3.0 mm

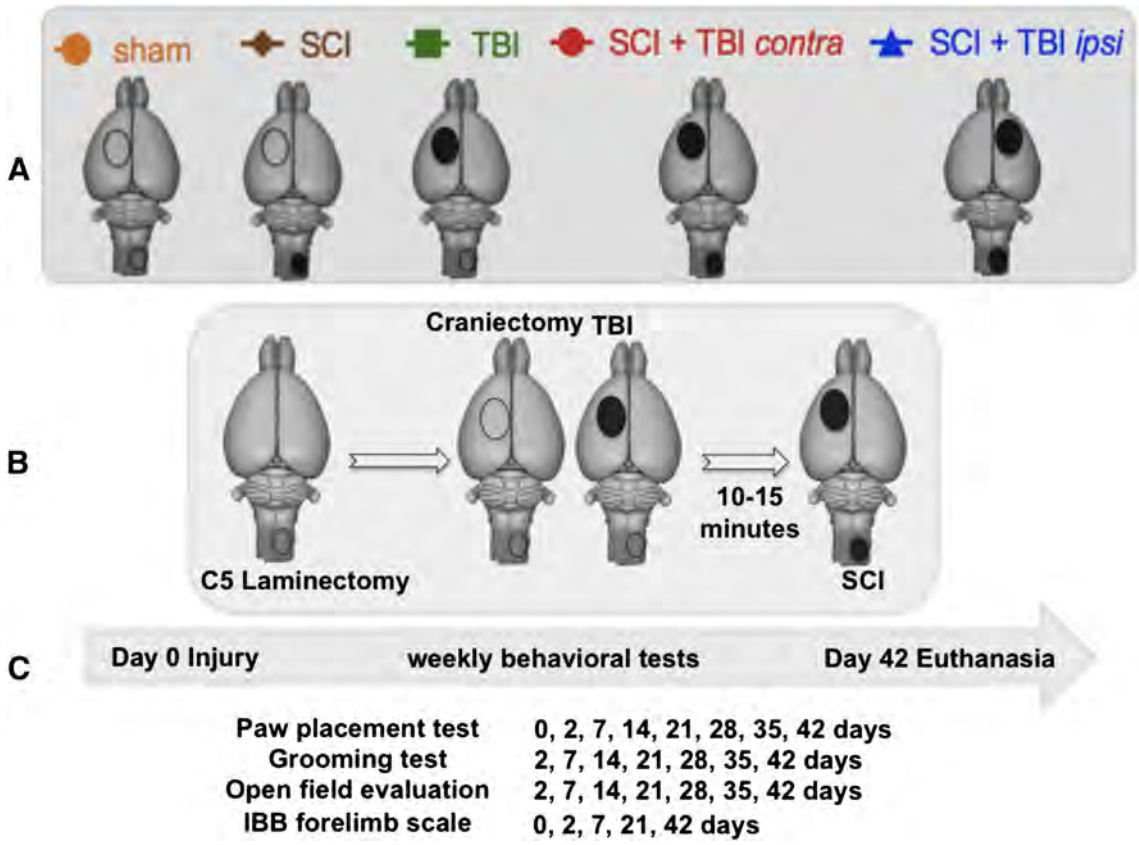


Fig. 1. (A) Injury groups: sham (craniectomy + laminectomy) (n = 9); spinal cord inury (SCI) + craniectomy (n = 10), traumatic brain injury (TBI) + laminectomy (n = 10), SCI + *contralateral* TBI (n = 10), SCI + *ipsilateral* TBI (n = 10). (B) Sequence of surgical procedures for producing the combined SCI + TBI model. (C) Behavioral testing was done 2, 7, 14, 21, 28, 35 and 42 days after the injury, and then rats were euthanized.

posterior and 3.0 mm anterior to bregma and between 1.0 and 7.0 mm lateral to bregma using a high-speed drill. CCI was produced using the electric CCI device with a 5.0 mm diameter impactor with a convex tip (Custom Design & Fabrication, Inc., Sandston, VA), which perpendicularly compressed the curvature of the sensori-motor cortex to a depth of 2.0 mm at 4.0 m/s velocity with a dwell time of 150 msec (Igarashi et al., 2007). The CCI machine was switched on when the top of the convex tip touched the pia. Contusion is one of the most common manifestations of head trauma in humans (Strich, 1970), making CCI a valuable and clinically relevant model of TBI. Heart rate and blood oxygenation was monitored with a Mouse Ox ™ pulse-oximeter (Torrington, Connecticut); temperature was monitored and maintained at 37.5 °C. The injury sites were closed and the animals were recovered in Thermocare®, Intensive Care Unit with Dome Cover (Thermocare, Inclined Village, NV).

Spinal cord injury model

We used the Infinite Horizon (IH) SCI device (Infinite Horizons, Inc., Lexington KY), which had a special impactor tip that was 2 mm in diameter (Scheff et al., 2003). Briefly a dorsal midline incision was made, the skin was dissected from underlying fascia, and the trapezius muscle was cut just lateral to the midline from C1/C2 to T2. Underlying muscle layers were blunt-dissected to expose the spinous processes from C3 to T1. A small animal retraction system (Fine Science Tools Inc., North Vancouver, BC, Canada) was used to hold muscle layers apart. A C5, dorsal laminectomy was performed to expose the entire right side and most of the left side of the spinal cord, leaving the dura matter intact. Rats were then secured in place with vertebral clamps at C4 and C6. The impactor rod was centered over the right side of the C5 laminectomy site with the medial edge of the 2 mm impounder aligned on midline to induce a unilateral injury similar to our earlier NYU/MASCIS device unilateral cervical contusion injuries (Gensel et al., 2006; Gruner, 1992; Mihai et al., 2008; Nout et al., 2009). Peak force was pre-set at 75 kdyn.

Behavioral testing

Behavioral testing was done by two observers blind to the lesion conditions, and rats were videotaped before surgery and at 2, 7, 14, 21, 28, 35 and 42 days after the injury (Fig. 1C). Motor performance was measured using the paw preference test (Dunham et al., 2010; Gensel et al., 2006; Schallert et al., 2000), the grooming test (Bertelli and Mira, 1993; Gensel et al., 2006), locomotion in an open field (Martinez et al., 2009), and the Irvine, Beatties, Bresnahan (IBB) cereal eating test (Irvine et al., 2010). All behavioral analyses were conducted by personnel blind to experimental group.

Paw preference test

Animals were placed in a clear plastic cylinder with two mirrors placed at angles such that both sides of the rat were clearly visible. The rats were recorded with a digital camera for 3 min, and slow motion high definition (HD) playback was used to determine the number of the times the animal placed its left, right, or both forepaws against the side of the cylinder during weight supported movements. Individual placements were scored as either “left” or “right” when 0.5 s or more passed without the other limb contacting the side of the cylinder. If both forepaws were used for weight-supported movements within 0.5 s of each other, a score of “both” was given. During lateral exploration, a “both” score was given for every two-step “walking” sequence, during which, both paws changed position on the side of the cylinder.

If one paw remained anchored while the other was placed on different parts of the cylinder, no score was given until the anchored forepaw was lifted. Scoring was performed using video playback by trained raters who were blind to experimental condition. Animals were tested before surgery and on day 2, 7, 14, 21, 28, 35 and 42 days after SCI.

Grooming test

This test was originally developed to examine recovery in a rat brachial plexus reconstruction model (Bertelli and Mira, 1993) and was adapted for cervical SCI by Gensel et al. (2006). Animals were placed in a clear plastic cylinder with two mirrors placed at angles such that the rat's head was always clearly visible. Cool tap water was applied to the animal's head and back with soft gauze prior to placing the animal in the cylinder, and the animal was recorded with a digital camera for 3 min. Slow motion HD playback was used to score each forelimb independently to identify the maximal contact made while performing any part of the grooming sequence. A six-point scoring system was used in which 0 indicates the animal is unable to contact any part of the face or head; 1 indicates the animal's forepaw makes contact with the underside of the chin and/or mouth area; 2 indicates the animal's forepaw contacts the area between nose and eyes, but not the eyes; 3 indicates the animal's forepaw contacts the eyes and the area up to, but not including, the front of the ears; 4 indicates the animal's forepaw contacts the ears, but not the area of the head behind the ears; and 5 indicates the animal's forepaw contacts the area of the head behind the ears. Animals were tested at 2, 7, 14, 21, 28, 35 and 42 days after injury.

Irvine, Beatties, Bresnahan (IBB) forelimb rating scale

Skilled forelimb function was assessed using an updated version of the IBB cereal eating test as described in Irvine et al. (2010). Briefly, rats were individually placed in their home cages and given spherical- and doughnut-shaped pieces of cereal that were of a consistent size and shape prior to the initiation of eating; rats were not scored when eating cereal pieces that were broken prior to testing initiation. Each trial was recorded to allow slow motion HD playback and evaluation of paw use. Videos of animals eating the cereal were evaluated using standardized scoring of common forelimb behaviors (including joint position, object support, digit movement and grasping technique) used while consuming both cereal shapes. An IBB score was assigned using the 10 point (0–9) ordinal scale for each shape, and the highest score i.e. the one reflecting the greatest amount of forelimb recovery, was assigned.

Open field locomotor test

The forelimb and hindlimb locomotor deficits were assessed during spontaneous locomotion using an open-field scoring derived from the Basso, Beattie and Bresnahan (BBB) scale (Basso et al., 1996) and designed for an accurate evaluation of the behavioral consequences of cervical SCI (Martinez et al., 2009). Rats were tested in pairs for a 4 min period in a circular plexiglas arena (95 cm diameter, 40 cm wall height) with an anti-skid floor. Open field locomotor function was assessed by two examiners, who consulted with one another to complete a scoring grid that gave a forelimb and hindlimb functional score to each animal in each session. Behavioral deficits affecting the limbs ipsilateral to the cervical SCI were categorized by evaluating the articular movement amplitude, weight support, fine distal positioning and stepping abilities. The scaling grid yielded final scores (maximum grade, 20) for the affected forelimb and hindlimb.

Histopathological analysis

At 42 days after surgery, animals were anesthetized with xylazine (TranquiVedTM, Vedco Inc., St. Joseph, MO; 10 mg/kg IP) and ketamine (ketamine HCl, Abbott Laboratories, N. Chicago, IL; 80 mg/kg IP), and transcardially perfused with 0.9% NaCl and 4% paraformaldehyde in phosphate-buffered saline (PBS). Brains and spinal cords were postfixed in 4% paraformaldehyde for 2 h and then cryoprotected in 30% sucrose in PBS for 48–72 h (until the tissue sank). The tissue was frozen at 80 °C until further analysis. The brains were sectioned transversely on a cryostat at 30 μm, and spinal cords at 20 μm. Sections were stained with hematoxylin and eosin. The remaining spared area of the brain at the epicenter, and at 2.5 mm anterior and 2.5 mm posterior from the center were calculated as a proportion of the ipsilateral brain at the each section. For the spinal cord, the remaining spared areas were measured as a proportion of the ipsilateral hemi-cord areas at the lesion epicenter. Areas of tissue damage were determined by the presence of large cystic cavities, aggregates of microcysts in the white matter, and dense gliosis. Motor neuron loss was assessed by counting large ventral horn motor neurons in sections 120 μm apart through 1.2 mm of cord centered on the lesion epicenter (10 sections per animal). All cells in the ventral horn ranging from 30 to 70 μm in diameter with a discernable nucleolus were counted.

Statistical analysis

Quantitative behavioral data and histopathological measurements are reported as mean ± standard error (SE) for each injury group. Factorial repeated measures analysis of variance (ANOVA) was used to analyze all behavioral data. For analyses that compared ipsilateral and contralateral limbs in the same animal, both time and limb were treated as repeated measures. The null hypothesis was rejected at $\alpha = 0.05$. Significant differences identified by the ANOVA were isolated using the Tukey's procedure for pairwise multiple comparison post-hoc test on group means in accordance with highly-cited best-practices from the statistical literature, (Keppel and Wickens, 2004). Spearman correlation was used to assess the relationship between behavior and histology. All statistics were performed with SPSS v.19 (IBM).

Upon publication of these primary data, all data will be entered into an NIH-supported centralized preclinical spinal cord injury database repository for later inclusion in advanced multivariate meta-analytic studies by the SCI research community (Ferguson et al., 2013).

Results

General health

All animals survived the full 42 days duration of the study. Subjects showed no obvious respiratory distress or bladder dysfunction at any time post injury and easily accessed water and food. There was a slight drop in body weight after surgery but this recovered in all groups by post-op day 14, and no animals dropped below 90% of pre-operative body weight during the post-operative period. No differences between groups were observed.

Behavioral results

All behavioral results are depicted over time (Figs. 2–4), and the full set of behavioral outcomes are shown as collapsed means in Fig. 4.

Paw placement test

The results on paw placement in the cylinder are shown in Fig. 2, and are reported as a percentage of either ipsilateral (right), contralateral (left), or simultaneous versus total paw placements. Some animals showed slight postural instability, but this did not inhibit their ability

to explore the cylinder. Pre-operatively, all animals used both paws simultaneously for the majority of weight supported wall movements in the cylinder (percentage of simultaneous vs. total placements 53.1 ± 1.7%; mean ± standard error of the mean (SEM)), and there was no significant overall preference for the use of either limb independently (left vs. total placements = 23.4 ± 1.4%; right vs. total 23.5 ± 1.5%; mean ± SEM).

The effects of SCI, TBI and the combination of these injuries is shown in Fig. 2. Simultaneous paw placements are shown in Fig. 2C, whereas independent ipsilateral (i.e. ipsilateral to the SCI, hence right) and contralateral (left) placement proportions are shown in Fig. 2B and D respectively. As can be seen in Fig. 2A, the patterns of recovery of paw use following the various lesions differed between groups. Use of the paw contralateral to the SCI (i.e. the left paw, solid bars in Fig. 2A) increased after SCI alone, TBI alone and especially after SCI + *contralateral* TBI. This additive effect is also evident in Fig 2D; compare SCI alone (brown line) to TBI alone (green line) to SCI + *contralateral* TBI (red line). Use of the paw ipsilateral to the SCI was almost abolished by the combined lesion (Fig. 2A gray and white, SCI + *contralateral* TBI; Fig. 2B, red line). Interestingly, adding an ipsilateral TBI to the SCI, reduced use of the left paw to near normal by week 1 following injury (Fig. 2A, black bars; Fig. 2D: compare the blue [SCI + *ipsilateral* TBI] and yellow [Sham] lines). And, independent and simultaneous use of the paw ipsilateral to the SCI (i.e. right forepaw) returned to a more equal balance with the left forepaw after injury (Fig. 2A, gray and white bars respectively; Fig. 2B blue line).

Statistical analysis of the scored results revealed a differential pattern of effects on the ipsilateral and contralateral limb, confirmed by 3-way, double repeated measures ANOVA (effect of injury condition with limb and time as repeated measures), $F = 6.08$, $p < 0.001$. Follow-up, 2-way repeated ANOVAs were performed for each limb separately. For the contralateral (left) paw placement data (Fig. 2D), a mixed repeated measures ANOVA revealed a significant main effect of injury condition, $F = 43.29$, $p < 0.0001$. In addition there was a significant effect of time and a time by injury condition interaction, all $F = 7.39$, all $p < 0.0001$. A posthoc Tukey's on the group means revealed that SCI + *contralateral* TBI was higher than either single injury group ($p < 0.05$; Fig. 4A). For the ipsilateral (right) paw placement data (Fig. 2B), mixed repeated measures ANOVA revealed a significant main effect of injury condition, $F = 18.52$, $p < 0.0001$. In addition there was a significant effect of time and a time by injury condition interaction, both $F > 2.92$, $p < 0.0001$. A Tukey's post hoc test on the group means revealed that SCI + *ipsilateral* TBI had less lateralization of function

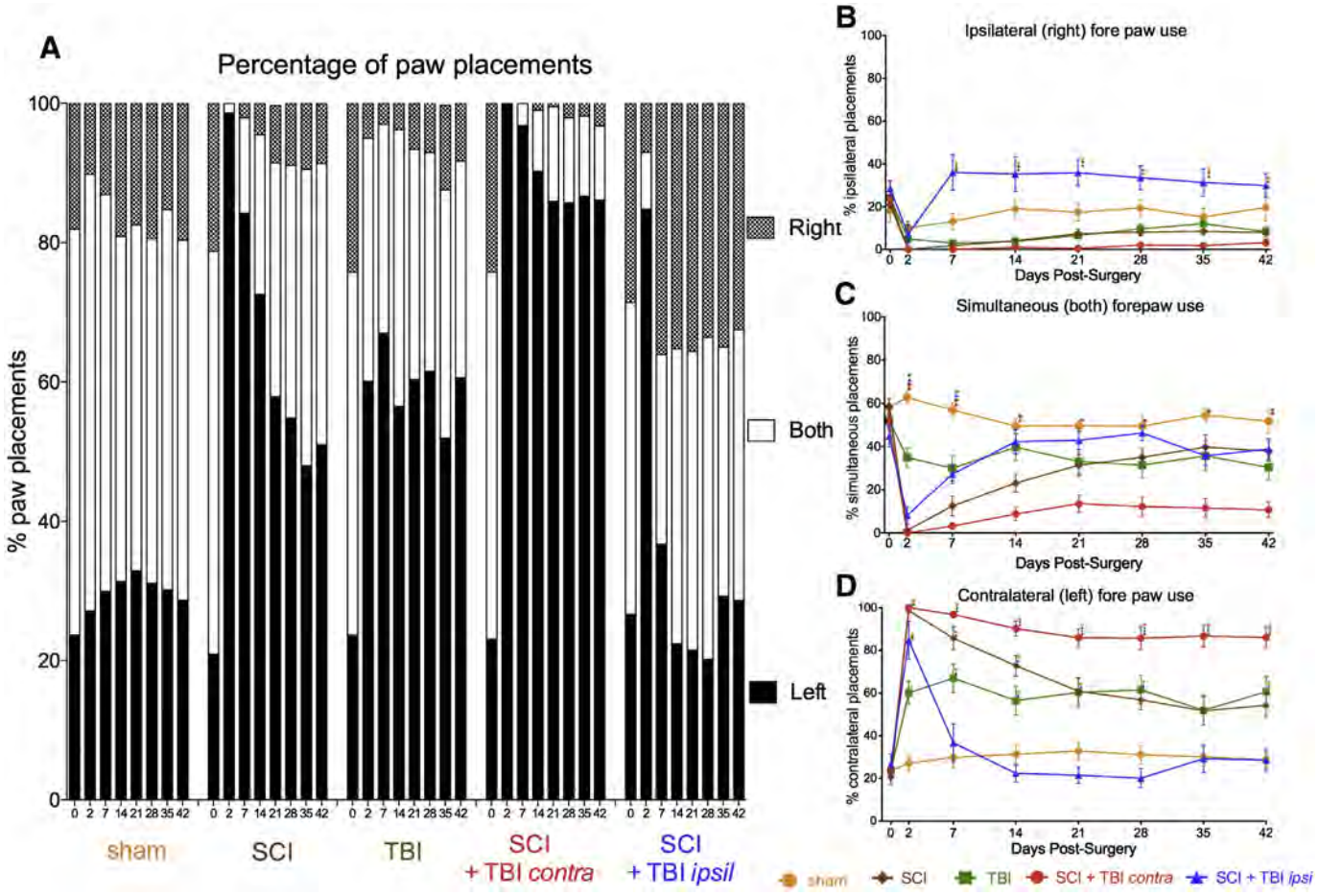


Fig. 2. Paw placements in the cylinder. (A) The percentage of total paw placements (left, right, or both) is shown for each group. (B) The proportion of total paw placements made by the ipsilateral (right) paw is shown. (C) The proportion of total paw placements made simultaneously by both paws is shown. (D) The proportion of total paw placements made by the contralateral (left) paw is shown. (A-D) The performance of sham rats did not significantly differ from pre-injury at any time post-operatively. SCI alone rats showed a profound deficit that recovered to the level of TBI alone rats over 42 days weeks. TBI produced a stable deficit in paw placement that did not recover over 6 weeks. SCI + *contralateral* TBI produced a profound deficit that failed to recover over 42 days, showing an almost complete preference for the limb contralateral to the SCI. SCI + *ipsilateral* TBI rats initially did not use the right forepaw (2 days after the injury) but they then significantly increased right limb use (i.e. ipsilateral to the SCI). (B) SCI + *ipsilateral* TBI significantly increased ipsilateral forepaw use compared to SCI + *contralateral* TBI rats 7 days after surgery. (C) SCI + *contralateral* TBI significantly reduced simultaneous forepaw use compared to sham. (D) SCI + *contralateral* TBI significantly enhanced contralateral forepaw use compared to both sham and SCI + *ipsilateral* TBI rats 7 days after surgery. SCI: spinal cord injury, TBI: traumatic brain injury, ○: significant difference compared to sham group, ◇:significant difference compared to SCI group, □:significant difference compared to TBI group, ▲:significant difference compared to SCI + *ipsilateral* TBI group.

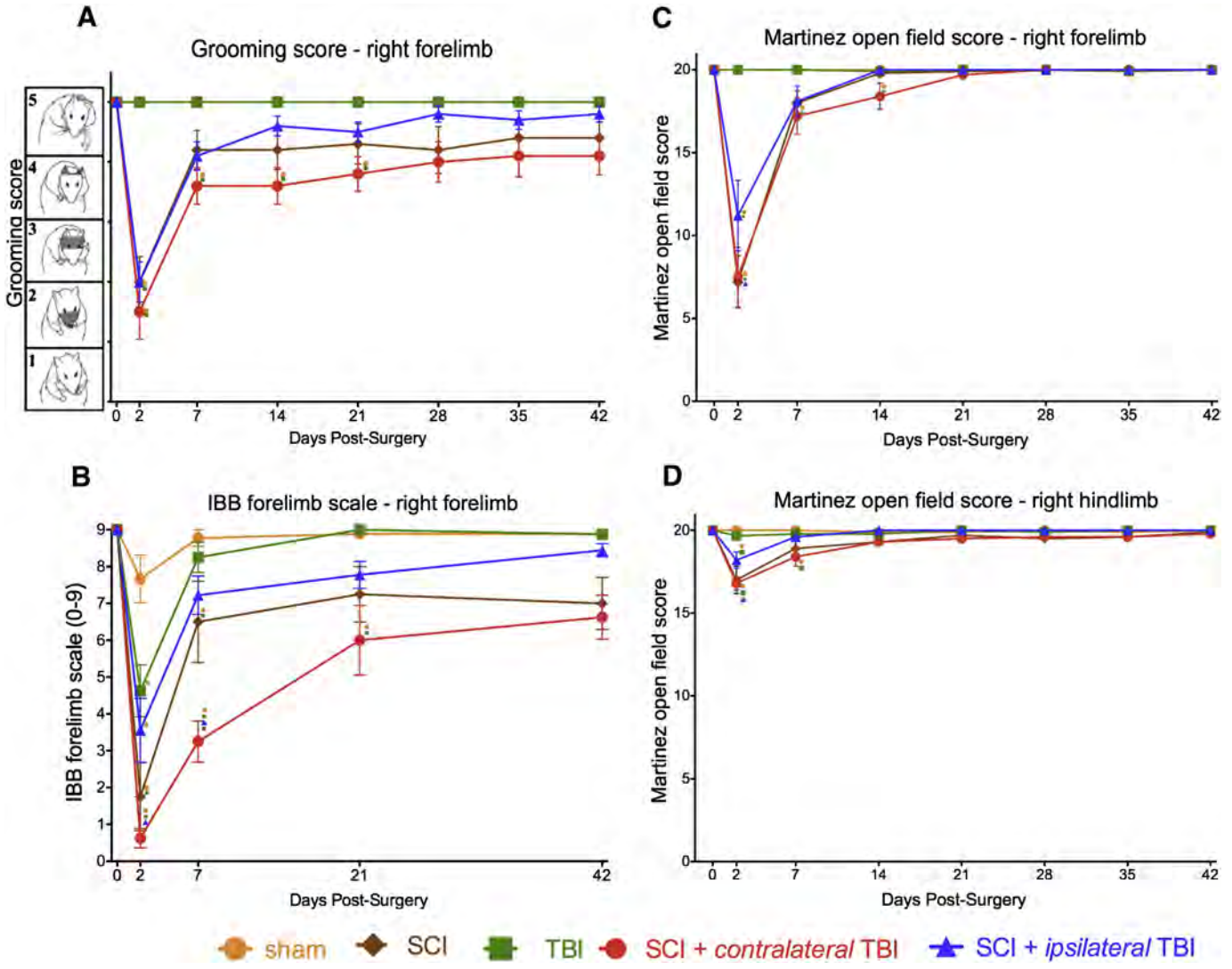


Fig. 3. Grooming, paw use (IBB) and open field locomotor performance over time after injury. (A) Both sham lesions and TBI alone did not affect grooming, but rats with SCI (SCI alone and combined SCI and TBI) produced deficits in grooming. Two days after injury, the majority of animals with SCI were only able to touch the bottom of the snout (score of 1), but they remarkably improved from 2 days to 7 days post-injury. Twenty-one days after the injury, only SCI + *contralateral* TBI rats demonstrated significantly lower grooming scores than sham and TBI only groups (score of 4). Forty-two days after injury, no statistically significant differences between groups were evident. (B) *Irvine Beatties Bresnahan (IBB) forelimb scale.* (Irvine et al., 2010). Before the injury, the rats could eat any type of cereal without any deficit in forelimb function (score of 9); but 2 days after the injury, rats with both SCI and TBI showed a significant decrease in ipsilateral (right) forepaw function. Seven days after the injury, only the SCI and SCI + *contralateral* TBI rats showed deficits in forelimb function. (C) Open field forelimb locomotor test using the Martinez score (Martinez et al., 2009). Neither sham nor TBI alone affected forelimb locomotor scores, but rats with SCI (SCI alone and combined SCI and TBI) exhibited deficits in locomotor function. Rats with SCI showed severe impairments of forelimb movements and postural abilities at 2 days post-injury but rapidly recovered within the first 7 days. SCI + *contralateral* TBI rats recovered more slowly than SCI alone and SCI + *ipsilateral* TBI rats, but finally reached a similar level of motor skills. (D) Openfield hindlimb locomotor test. During the first 7 days after the injury, rats with SCI showed hindlimb deficits, which were mainly characterized by poor stepping. SCI: spinal cord injury, TBI: traumatic brain injury, ○: significant difference compared to sham group, □: significant difference compared to TBI group, ▲:significant difference compared to SCI + *ipsilateral* TBI group.

than the other injuries ($p < 0.05$; Fig. 4B). Analysis of the raw response numbers demonstrated that the SCI + *ipsilateral* TBI had a significantly lower overall number of responses than SCI alone, $p < .05$; no other group differences were significant, $p > .05$ (Supplementary Fig. 1). To test the extent to which the change in overall response number influenced the lateralization results we used analysis of covariance (ANCOVA) to correct for total response number. Response number was not a significant covariate for either right or left paw preference, both $p > .05$, indicating that response number did not significantly influence the results (Supplementary Fig. 2). Together, the statistical results indicated that both SCI and *contralateral* TBI produced a lateralization of paw preference that was additive in the combined injury condition. However, subjects with SCI + *ipsilateral* TBI had a more balanced ipsilateral and contralateral forelimb use.

Grooming test

All animals showed normal grooming with the contralateral (left) forelimb at all time-points post-injury (data not shown). TBI alone did not affect grooming performance (Fig. 3A, green line), but rats with SCI (either SCI alone or combined SCI and TBI) showed significant deficits in grooming (Fig. 3A, brown, red and blue lines). Two days after injury, the majority of animals with SCI were only able to touch the bottom of the snout (score of 1), but improved from day 2 to 7 post-injury (SCI alone, 4.2 ± 0.28, SCI + *contralateral* TBI, 3.6 ± 0.30, SCI + *ipsilateral* TBI; 4.1 ± 0.23; mean ± SEM). By twenty-one days after the injury, only the SCI + *contralateral* TBI group exhibited sustained deficits in the ability to groom (SCI + *contralateral* TBI, 4.0 ± 0.33; mean ± SEM).

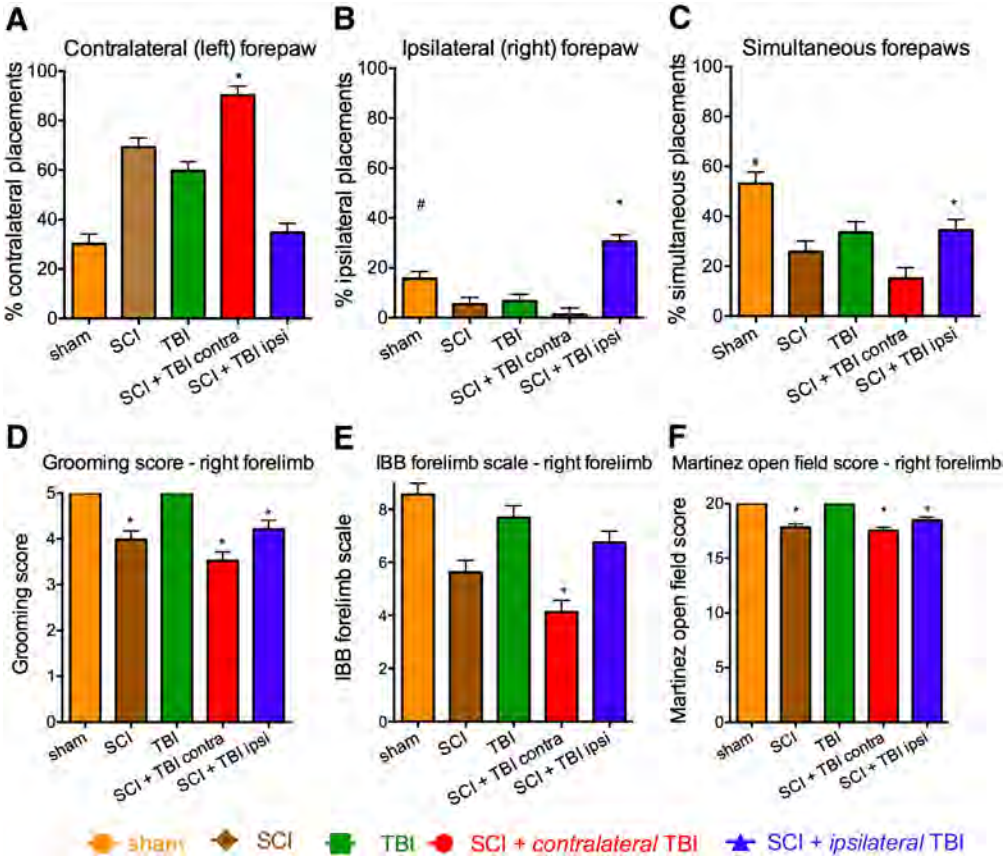


Fig. 4. Performance over all days. (A) *Contralateral (left) forepaw placements*: The SCI + *contralateral* TBI group used their contralateral (left) forepaw more than other injury groups ($p^* < 0.05$). (B) *Ipsilateral (right) forepaw placements:* SCI + *ipsilateral* TBI rats used their right forepaw more as compared to the other injury groups ($p^* < 0.05$). The Sham group used the ipsilateral forepaw more than SCI alone, TBI alone, and SCI + *contralateral* TBI ($p\# < 0.05$). (C) Simultaneous (both) paw placements: Shams used simultaneous forepaw placements more than the injury groups ($p\# < 0.05$). SCI + *ipsilateral* TBI group used simultaneous forepaw placement more than SCI + *contralateral* TBI group ($p^* < 0.05$). (D) *Grooming test:* Sham and TBI alone groups had better grooming scores than groups with SCI ($p^* < 0.05$). (E) *Irvine, Beatties, Bresnahan (IBB) forelimb scale:* The SCI + *contralateral* TBI group had worse performance than other injury groups ($p^* < 0.05$) (F) *Open field forelimb locomotor test:* Sham and TBI alone groups performed better than SCI alone, SCI + *contralateral* TBI, and SCI + *ipsilateral* TBI groups ($p^* < 0.05$). SCI: spinal cord injury, TBI: traumatic brain injury. Error bar shows standard error of the mean.

Statistical analysis revealed a differential effect of TBI and SCI on grooming. A 2-way mixed repeated measures ANOVA revealed a significant main effect of injury condition, $F = 11.40$, $p < 0.0001$. In addition there was a significant effect of time and a time by injury condition interaction, both $F > 9.77$, $p < 0.0001$. A posthoc Tukey's performed on the group means revealed that Sham and TBI alone were better than SCI alone, SCI + *contralateral* TBI, and SCI + *ipsilateral* TBI groups ($p < 0.05$; Fig. 4D), but the groups with SCI did not differ from each other. The results confirm that SCI, but not TBI produced a deficit on the grooming task.

Irvine Beatties Bresnahan (IBB) forelimb scale

The IBB cereal eating test was employed to evaluate injury effects on skilled forelimb function and digital control (Irvine et al., 2010). After injury while eating either the doughnut or spherical shaped cereals, the impaired forelimb was engaged for food manipulation. At 2 days after the injury, both SCI and TBI alone produced a significant impairment in ipsilateral (right) forepaw function compared to sham injuries (Fig. 3B).

Statistical analysis confirmed these effects. A 2-way mixed repeated measures ANOVA revealed a significant main effect of injury condition, $F = 15.74$, $p < 0.0001$. In addition there was a significant effect of time and a time by injury condition interaction, all $F = 6.90$, all $p < 0.0001$. A posthoc Tukey's test performed on the group means revealed that SCI + *contralateral* TBI was significantly worse than all groups ($p < 0.05$), except for SCI alone ($p = .14$) (Fig. 4E). To test for transient deficits, we performed further post-hoc testing of early time-points. We found that rats in the SCI + *contralateral* TBI group had significantly poorer ipsilateral forepaw use than the SCI + *ipsilateral* TBI group on 2 and 7 days after injury. The SCI alone group fell consistently between the SCI + *contralateral* TBI and SCI + *ipsilateral* TBI groups, but only differed significantly from the SCI + *contralateral* TBI group on 7 days after injury (Fig. 3B).

Forelimb and hindlimb open field locomotor tests

To evaluate forelimb and hindlimb locomotor function we used the scaling system described by Martinez et al. (2009). TBI alone affected neither forelimb nor hindlimb locomotor tests, but rats with SCI (SCI alone and combined SCI and TBI) had locomotor deficits. Qualitative patterns were as follows:

1. Forelimb function recovery. Rats with SCI showed severe impairments of forelimb movements and postural abilities at 2 days post-injury (Fig. 3C). The injuries lead to a flaccid paralysis of the forelimb, characterized by a lack of movements of the distal joints and restricted movements of the proximal ones. These impairments resulted in a lack of postural support. Rats with SCI alone exhibited the most

prominent and rapid recovery of the affected forelimb locomotor abilities within the first 7 days. SCI + *contralateral* TBI rats recovered more slowly than SCI alone and SCI + *ipsilateral* TBI rats, but all recovered.

Statistical analysis confirmed these results. A 2-way mixed repeated measures ANOVA revealed a significant main effect of injury condition, $F = 16.44$, $p < 0.0001$. In addition there was a significant effect of time and a time by injury condition interaction, both $F > 14.74$, all $p < 0.0001$. Posthoc Tukey's test of the group means revealed that Sham and TBI alone groups performed better than SCI alone, SCI + *contralateral* TBI, and SCI + *ipsilateral* TBI groups ($p < 0.05$; Fig. 4F).

2. *Hindlimb function recovery.* During the first 7 days after the injury, rats with SCI showed moderate hindlimb deficits, which were mainly characterized by poor stepping (Fig. 3D). Rats with SCI alone (19.1 ± 0.88, Martinez scale) and SCI + *contralateral* TBI (19.0 ± 0.38. Martinez scale) showed a similar pattern of loss and recovery. SCI + *ipsilateral* TBI produced significantly better recovery than SCI + *contralateral* TBI rats within the first 7 days ($p < 0.05$), and also recovered slightly but not significantly better than SCI alone ($p > 0.05$). A 2-way mixed repeated measures ANOVA revealed a significant main effect of injury condition, $F = 8.43$, $p < 0.0001$. In addition there was a significant effect of time and a time by injury interaction, both $F > 4.76$, all $p < 0.0001$. A posthoc Tukey's test on the group means revealed that the SCI + *contralateral* TBI group were worse than Sham, TBI alone, and SCI + *ipsilateral* TBI groups ($p < 0.05$). The SCI alone was worse than TBI alone and sham groups ($p < .05$).

Histological outcomes

Spinal cord and brain lesion extents are depicted for the median size of the lesions with TBI (Fig. 5A) and the groups with SCI (Fig. 5B). There were no statistically significant differences in the size or location of the lesions between injury groups. Statistical analysis of the TBI histology at lesion epicenter using oneway ANOVA and Tukey's posthoc tests revealed that the TBI alone, SCI + *ipsilateral* TBI and SCI + *contralateral* TBI + groups had lower brain sparing than either SCI or sham groups, $F = 44.75$, $p < .0001$. The size of the TBI lesions did not differ between groups with TBI, all $p > .05$ (Fig. 5C). Analysis of spinal cord sparing using oneway ANOVA and Tukey's posthoc tests showed a significantly lower sparing in SCI alone, SCI + *ipsilateral* TBI, and SCI + *contralateral TBI* relative to TBI alone and sham, $F = 44.03$, $p < .0001$. However there were no significant differences between the groups with SCI, all $p > .05$ (Fig. 5D). Analysis of motor neuron number was performed by 2-way repeated measures ANOVA (distance from epicenter as a repeated measure). Motor neuron counts throughout the extent of the lesion are shown in Fig. 5E. Groups with SCI also showed significant effect of group by distance on motor neuron loss, relative to Sham and TBI groups, $F = 6.27$, $p < .0001$ (Fig. 5E; SCI groups vs. sham, $p < 0.05$; SCI groups vs. TBI, $p < 0.05$).

Behavior and histology

To evaluate the relationship between behavior and histology we used Spearman correlations (Table 1). Brain lesion sparing significantly predicted performance on paw preference but not other tasks. Left-sided brain sparing was inversely correlated with left limb use and positively correlated with right limb use. The converse pattern was observed for right-sided brain sparing. In contrast, the right-sided spinal cord injury lesion size did not generate systematic lateralization in the full combined injury dataset correlating with the number of bilateral paw placements but not the left and right limbs individually. It should be noted that spinal cord lesion size correlated with other bilateral outcome tasks, including grooming, IBB, forelimb openfield, and hindlimb openfield performance.

Of the functional measures taken, only the total number of bilateral placements correlated with both brain and spinal cord sparing. In particular left-sided brain sparing and right-sided spinal cord sparing both positively correlated with bilateral paw placement. Together, the correlational analysis reinforces the concept that in combined SCI + TBI, an activated left hemisphere helps compensate laterality in paw preference that is produced by an intact left hemisphere.

Discussion

In light of the clinical evidence that SCI is often accompanied by TBI 'dual diagnosis' (Macciocchi et al., 2008), the principle purpose of the present study was to begin the development of a rat preclinical model of combined TBI and SCI. For practical reasons as well as experimental design concerns, we chose to employ a well-characterized unilateral cervical contusion injury (Gensel et al., 2006) and a controlled cortical impact injury (Igarashi et al., 2007). We were able to determine the effects of individual and combined injuries on a variety of neurological outcome measures focused on forelimb function. In addition, this paradigm revealed a laterality of TBI effect that we suggest reflects underlying dynamic mechanisms of neurological dysfunction that have important implications for repair and rehabilitation after CNS injuries.

Effects of SCI or TBI alone

SCI and TBI alone each produced significant changes in paw placement preference, as predicted from prior work (Gensel et al., 2006; Schallert et al., 2000). As expected, this occurred ipsilateral to the right-sided SCI only lesion, and contralateral to the CCI-only lesion. The moderate SCIs used in this study produced a profound initial lack of use of the ipsilateral (right) forepaw in the cylinder, but this recovered substantially over the 6 weeks of the post-injury period. The CCI-TBI injury produced somewhat of a lesser decline in paw placement by the right (contralateral to the lesion), but this decline was permanent over the post-operative course. The grooming test showed quite different results. As predicted by prior work (Gensel et al., 2006), the 75 kdyne IH unilateral cervical injury produced a moderate deficit in the ability of the rats to use their right (ipsilateral) forelimb to reach the back of the head during grooming, and this ability recovered somewhat over the 6 weeks after SCI. CCI-TBI on the contralateral (left) side, which damages cortical and other projections to the spinal cord controlling the right (ipsilateral) forelimb, produced essentially no deficit at all in right sided (or left sided) grooming. Thus, performance on the grooming test must reflect abilities mediated by the circuits damaged by the cervical SCI, but not by those damaged by the CCI-TBI. The IBB cereal test was sensitive to both injuries, and recovered partially after moderate SCI alone, and almost completely after TBI alone. The open field locomotor test was only modestly and transiently affected by either lesion alone. The differences in sensitivity of these outcome measures to SCI vs. TBI, and the differences in degree and rate of recovery, no doubt reflect differences in the neural substrates underlying these behaviors and their recoveries after CNS injury.

Based on substantial evidence for an important role of the forebrain and cortex in mediating plasticity and recovery of function after SCI, we reasoned that a concurrent TBI using the cortical contusion injury method would have deleterious effects on neurological outcome and recovery when compared to SCI alone. In rats receiving a TBI contralateral to the SCI, this rather obvious prediction was strongly supported by the data, although the degree of interaction between TBI and SCI varied depending upon the measure used. A contralateral (left) TBI given at the same time as the right-sided SCI produced a profound reduction in ipsilateral paw placement, with

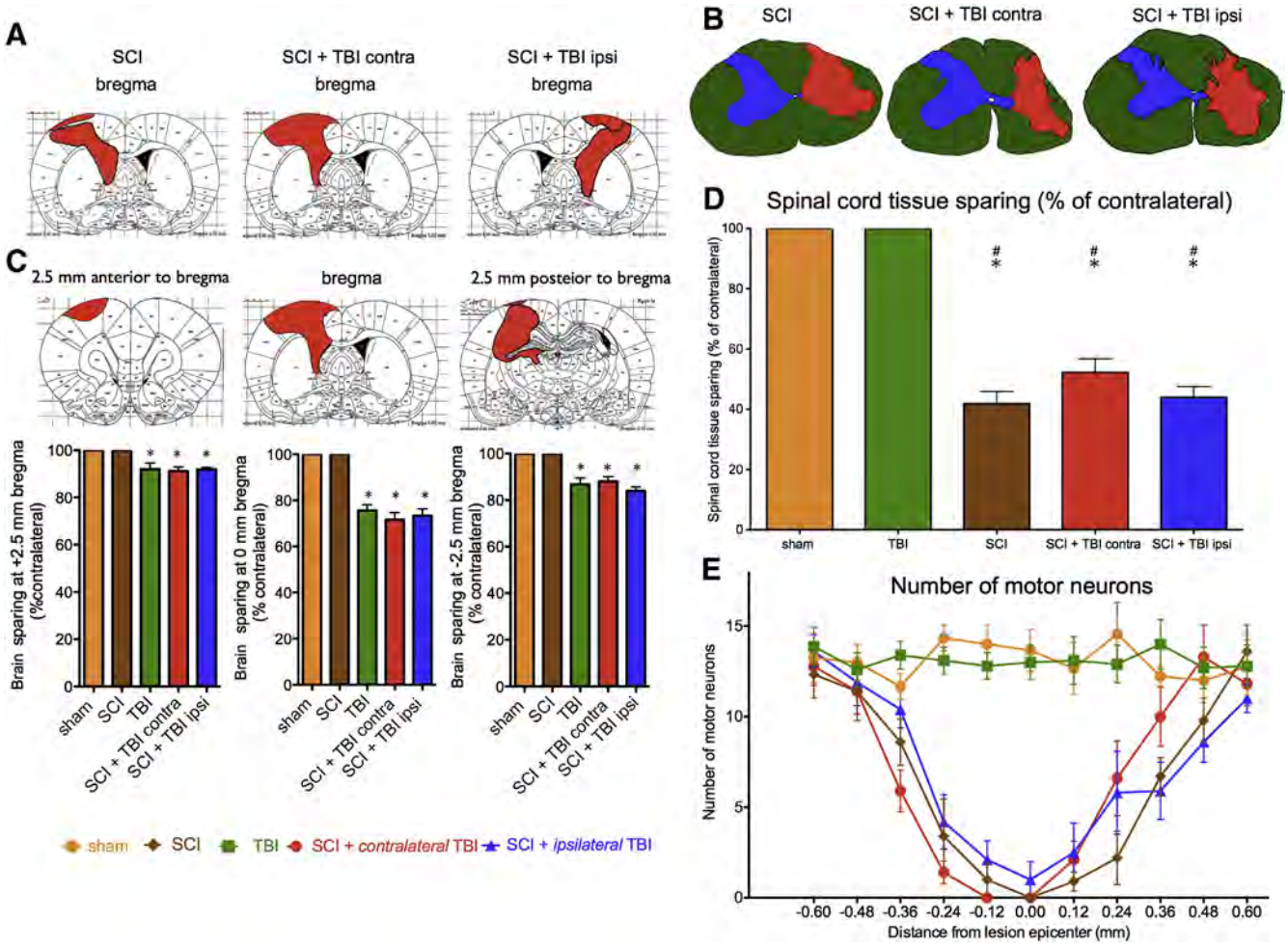


Fig. 5. Both brain (A) and spinal cord (B) lesions were not significantly different in size or location between groups. The median lesions are shown for each group (A,B), and for each location.. (C). The median tissue sparing at 2.5 mm anterior to bregma, at bregma, and 2.5 mm posterior to bregma is shown below (*p < 0.05 vs. Sham or SCI alone). (D) Spinal cord sparing at the epicenter of the lesion is shown (#p < 0.05 vs. sham; *p < 0.05 vs. TBI). (E) Motor neuron counts throughout the extent of the lesion show no significant differences between injury groups. SCI: spinal cord injury, TBI: traumatic brain injury.

almost all paw placements in the cylinder being made with *only* the left paw. The combination lesion resulted in a sustained and more significant deficit than either SCI or TBI alone. This is not surprising given the evidence that SCI and CCI produce paw placement deficits by somewhat different mechanisms (i.e. 'lower motor' spinal deficits and 'cortical sensorimotor neglect') (Schallert and Woodlee, 2003).

Table 1
Spearman correlation (r_s) between behavior test and tissue sparing.

	Tissue sparing at lesion epicenter		
	Spinal cord	Brain	
	Right	Left	Right
	r_s	r_s	r_s
Paw Preference, Left	−0.31**	−0.56 ***	0.41 *
Paw Preference, Right	–	0.46 **	−0.61 ***
Paw Preference, Both	0.43 **	0.34 *	–
Grooming	0.85 ***	–	–
Cereal	0.54 ***	–	–
Forelimb Openfield	0.84 ***	–	–
Hindlimb Openfield	0.60 ***	–	–

–: not significant
* p < .01.
** p < .001.
*** p < .0001.

In addition, plasticity in cortical/forebrain mechanisms is likely to be involved in mediating recovery of function. While contralateral (left) CCI-TBI injuries alone had no effect on the grooming response, they appeared to retard the time course and degree of recovery from a right-sided SCI (Fig. 4). Similarly, the combined SCI plus a contralateral CCI-TBI produced an enhanced initial deficit and retarded recovery on the IBB cereal eating measure, and on the initial recovery of the Martinez open-field score. Thus, the addition of a contralateral CCI-TBI appeared to be additive to the SCI-produced deficits for each of the forelimb tests used, but perhaps with different degrees of effects. This all provides additional evidence suggesting that contralateral cortical/forebrain plasticity is involved in recovery of forelimb function after unilateral cervical SCI. Note that there is also evidence for a role of the spared, ipsilateral cortex in recovery after unilateral cervical SCI (Rosenzweig et al., 2010; Strong et al., 2009).

The initial rationale for including a group with an ipsilateral (right sided) CCI-TBI was to provide a CNS injury control that would evaluate the effects of loss of forebrain-cortical systems not directly involved in the mediation of the movements used to measure recovery. However, examination of the effects of the ipsilateral CCI-TBI on the paw placement measure reveals effects that suggest interactions between the two sides of the brain after unilateral spinal cord or brain injuries. SCI alone results in a nearly complete loss of use of the ipsilateral right paw on day 2, and a recovery on day 7 to less than 20% (combining the independent and 'both' columns, Fig. 2A). Adding a contralateral

CCI-TBI makes this even worse, with almost no recovery over 6 weeks. However, adding a CCI-TBI to the *ipsilateral* brain results in less of a deficit at 2 days, and a return to a more normal balanced proportion of ipsilateral and contralateral forepaw use by 7 days, continuing until 6 weeks. Thus, the ipsilateral CCI-TBI appears to both reduce the initial SCI deficit and promote equal use of the forepaws compared to SCI alone, while the contralateral CCI-TBI produces a profound worsening of the deficit.

What can explain the apparent release of function by an ipsilateral brain injury? How can additional damage enhance performance and recovery? Inhibitory interactions between ipsilateral and contralateral cortical and subcortical structures have been described in a number of experiments over the years. For example, complete ablation of the striate and extrastriate cortex in cats produces a profound and apparently permanent visual hemianopsia resulting in lack of orientation to visual stimuli in the contralateral visual field. However, ablation of the contralateral superior colliculus 'released' visual orientation immediately, suggesting that the cortical lesion had produced an inhibition of the ipsilateral colliculus that masked the residual capacity for orientation (Sprague, 1966). Damage to the cortex in humans and animals, induces a 'compensatory reliance' on the ipsi-lesional limb (Jones et al., 2012). In addition, unilateral cortical damage actually appears to *enhance* the skilled reaching ability of the ipsi-lesional limb (Hsu and Jones, 2005, 2006; Jones et al., 1996, 2009, 2012; Luke et al., 2004), and this increased motor skill is accompanied by evidence of plasticity, including increased numbers of synapses on neurons in the contra-lesional cortex. Humans with cortical damage, especially on the parietal regions of the right side, exhibit a contralateral spatial inattention (or neglect) that appears to reflect a lack of awareness rather than an inability to see or move on the contralateral side. Whether our results depend upon contralateral inattention or ipsilateral enhancement, or how these effects are related, will require additional experiments. However, it may be reasoned that ipsilateral enhancement and contralateral neglect after cortical lesions or TBI are related by cortical plasticity and synaptogenesis (Hsu and Jones, 2005, 2006; Jones et al., 2009, 2012).

Deficits in paw placement in the cylinder have been seen repeatedly with cortical injuries, and have been ascribed to 'sensorimotor neglect' (Schallert et al., 2000). Thus, cortical TBI lesions by themselves may reduce the use of the forepaw in the cylinder test by reducing the initiation or production of the paw movement without necessarily disrupting the motor apparatus needed to perform the movement. This is in contrast to the complete lack of effect of TBI/cortical damage on the grooming response, which nevertheless recruits activation and coordination of some of the same spinal motoneuron pools and muscles involved in paw placement (McKenna et al., 2000). The C5 contusion lesion, on the other hand, is located in a position to both reduce motoneuron and spinal circuits directly involved in lifting the limb and partially damages corticospinal and other descending tracts rostral to distal forelimb motoneuron pools, and therefore could be expected to reduce paw placement and grooming function by a combination of effects on both. Both paw placement and grooming show partial recovery after unilateral SCI alone, and this likely reflects reorganization and plasticity at the spinal level as well as in the brain. The release of ipsilateral paw placement by the ipsilateral cortical TBI suggests that the residual capacity for lifting and placing the paws is relatively preserved after the C5 contusion (vertebral level), and that part of the deficit seen after a unilateral cervical SCI alone is due to damage to descending (e.g. corticospinal) fiber tracts. Thus, the superimposition of a contralateral inattention or neglect by the ipsilateral cortical lesion might release, or force, the residual capacity of the partially damaged ipsilateral cord. This view suggests that the cortical lesion is 'dominant' in this particular combination of CNS deficits, and raises the possibility that the release of, or altered balance, of circuits due to combined lesions might in some cases actually provide enhanced recovery. The corollary for rehabilitation strategies is that suppression of activity from unbalanced inputs might be useful for improving function, such as is seen in forced-use protocols after stroke (Willis et al., 2002; Wolf et al., 1989).

While no studies of concurrent SCI and TBI in rodent models seem to exist, there are a number of relevant studies of sequential lesion effects that also point to important interactions between brain and spinal circuits in recovery (Blanco et al., 2007). Blanco et al. (2007) measured grip strength after unilateral cervical hemisections or sensorimotor cortex lesions in the mouse. Recovery of grip strength occurred over several weeks after either SCI or cortex lesions (compare to the current data from paw placement or IBB score). Recovery after SCI was reversed by contralateral cortical lesions given 26–28 days after the initial injury, suggesting that the contralateral cortex had compensated for the loss of gripping ability from SCI. Lesions of the cortex ipsilateral to the original spinal hemisection did not reverse recovery of the ipsilateral cord deficit. But unexpectedly, those lesions also did not result in any impairment of grip strength of the contralateral forepaw. Thus, the recovery of function from spinal hemisection had somehow induced the capacity to mediate gripping to either the contralateral cortex or to subcortical (or even spinal) circuits. While these experiments involved sequential lesions that allowed time for compensatory plasticity to occur, the issues of how such a transfer takes place, e.g. through forced practice caused by inability or inattention, are similar to those raised by the current findings. One might speculate that the forced practice of the contralateral forepaw due to disuse of the paw ipsilateral to the spinal hemisection provided activity-dependent plasticity sufficient to free the left forelimb from dependence on cortical inputs.

Other work showing that activity in the non-impaired forelimb may actively suppress recovery of the contralateral impaired limb after cortical damage provides additional evidence for the presence of inhibitory or 'unbalanced' activity-dependent plasticity that might provide a target for suppressive strategies in rehabilitation (Allred et al., 2010; Bury and Jones, 2004). Indeed, Bury and Jones (2004) provide another example of improving function by inducing CNS damage, in their case, by cutting the corpus callosum to release the contralateral limb from the deleterious effects of activity induced plasticity of the undamaged cortex.

Thus, the concurrent combined injury model investigated in this study provides a first step towards evaluating interactions between TBI and SCI in a preclinical model. While it is acknowledged that purely unilateral CNS traumatic injuries are not common, especially in SCI, this approach has yielded some dramatic evidence of the complexity associated with recovery from dual injuries. Along with previous work on unilateral brain damage and recovery, the findings suggest that therapies for combined injury will need to consider complex interrelations between injuries and treatments to optimize adaptive, and minimize maladaptive, plasticity in recovery (Huie et al., 2012). Treatments, for example drugs, that positively affect recovery from TBI could adversely affect recovery from SCI, and vice versa, and, the added complication of the problem of balancing inputs after injury provides a strong impetus to continue development of preclinical models to inform clinical practice.

Conclusion

The current study provides strong evidence for complex interactions between SCI and TBI that affect recovery of forelimb function in a model of concurrent combined unilateral injuries in the rat. These data first support the idea that brain damage should exacerbate deficit and reduce recovery after SCI, but add the caveat that balanced activity and potential inhibitory effects of remaining systems will need to be considered in planning for treatments of 'dual diganosis' patients.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.expneurol.2013.06.006.

Acknowledgment

This work was supported by DoD/CDMRP SCIRP Translational Partnership Award #W81XWH-10-1-0910. T. Inoue was supported by the Uehara Memorial Foundation Research fellowship. The authors would like to acknowledge Ms. Ellie Stuck and Yvette Nout, DVM, PhD for help with some of the behavioral testing, and Jeffrey Sacramento and Jinghua Yao for help with the histological preparations and analysis.

References

Allred, R.P., Cappellini, C.H., Jones, T.A., 2010. The "good" limb makes the "bad" limb worse: experience-dependent interhemispheric disruption of functional outcome after cortical infarcts in rats. Behav. Neurosci. 124, 124–132.

Arzaga, D., Shaw, V., Vasile, A.T., 2003. Dual diagnoses: the person with a spinal cord injury and a concomitant brain injury. SCI Nurs. 20, 86–92.

Basso, D.M., Beattie, M.S., Bresnahan, J.C., 1996. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. Exp. Neurol. 139, 244–256.

Bell, R.S., Vo, A.H., Neal, C.J., Tigno, J., Roberts, R., Mossop, C., Dunne, J.R., Armonda, R.A., 2009. Military traumatic brain and spinal column injury: a 5-year study of the impact blast and other military grade weaponry on the central nervous system. J. Trauma 66, S104–S111.

Bertelli, J.A., Mira, J.C., 1993. Behavioral evaluating methods in the objective clinical assessment of motor function after experimental brachial plexus reconstruction in the rat. J. Neurosci. Methods 46, 203–208.

Blanco, J.E., Anderson, K.D., Steward, O., 2007. Recovery of forepaw gripping ability and reorganization of cortical motor control following cervical spinal cord injuries in mice. Exp. Neurol. 203, 333–348.

Bradbury, C.L., Wodchis, W.P., Mikulis, D.J., Pano, E.G., Hitzig, S.L., McGillivray, C.F., Ahmad, F.N., Craven, B.C., Green, R.E., 2008. Traumatic brain injury in patients with traumatic spinal cord injury: clinical and economic consequences. Arch. Phys. Med. Rehabil. 89, S77–S84.

Bury, S.D., Jones, T.A., 2004. Facilitation of motor skill learning by callosal denervation or forced forelimb use in adult rats. Behav. Brain Res. 150, 43–53.

Cooper, D.J., Ackland, H.M., 2005. Clearing the cervical spine in unconscious head injured patients - the evidence. Crit. Care Resusc. 7, 181–184.

Davidoff, G., Morris, J., Roth, E., Bleiberg, J., 1985a. Closed head injury in spinal cord injured patients: retrospective study of loss of consciousness and post-traumatic amnesia. Arch. Phys. Med. Rehabil. 66, 41–43.

Davidoff, G., Morris, J., Roth, E., Bleiberg, J., 1985b. Cognitive dysfunction and mild closed head injury in traumatic spinal cord injury. Arch. Phys. Med. Rehabil. 66, 489–491.

Davidoff, G., Roth, E., Morris, J., Bleiberg, J., Meyer Jr., P.R., 1986. Assessment of closed head injury in trauma-related spinal cord injury. Paraplegia 24, 97–104.

Davidoff, G., Thomas, P., Johnson, M., Berent, S., Dijkers, M., Doljanac, R., 1988. Closed head injury in acute traumatic spinal cord injury: incidence and risk factors. Arch. Phys. Med. Rehabil. 69, 869–872.

Dennis, A.M., Haselkorn, M.L., Vagni, V.A., Garman, R.H., Janesko-Feldman, K., Bayir, H., Clark, R.S., Jenkins, L.W., Dixon, C.E., Kochanek, P.M., 2009. Hemorrhagic shock after experimental traumatic brain injury in mice: effect on neuronal death. J. Neurotrauma 26, 889–899.

Dunham, K.A., Siriphorn, A., Chompoopong, S., Floyd, C.L., 2010. Characterization of a graded cervical hemicontusion spinal cord injury model in adult male rats. J. Neurotrauma 27, 2091–2106.

Ferguson, A.R., Irvine, K.A., Gensel, J.C., Nielson, J.L., Lin, A., Ly, J., Segal, M.R., Ratan, R.R., Bresnahan, J.C., Beattie, M.S., 2013. Derivation of multivariate syndromic outcome metrics for consistent testing across multiple models of cervical spinal cord injury in rats. PLoS One 8 (3), e59712 (PMID: 23544088).

Gensel, J.C., Tovar, C.A., Hamers, F.P., Deibert, R.J., Beattie, M.S., Bresnahan, J.C., 2006. Behavioral and histological characterization of unilateral cervical spinal cord contusion injury in rats. J. Neurotrauma 23, 36–54.

Gruner, J.A., 1992. A monitored contusion model of spinal cord injury in the rat. J. Neurotrauma 9, 123–126 (discussion 126–128).

Hagen, E.M., Eide, G.E., Rekand, T., Gilhus, N.E., Gronning, M., 2010. Traumatic spinal cord injury and concomitant brain injury: a cohort study. Acta Neurol. Scand. Suppl. 51–57.

Holly, L.T., Kelly, D.F., Counelis, G.J., Blinman, T., McArthur, D.L., Cryer, H.G., 2002. Cervical spine trauma associated with moderate and severe head injury: incidence, risk factors, and injury characteristics. J. Neurosurg. 96, 285–291.

Hsu, J.E., Jones, T.A., 2005. Time-sensitive enhancement of motor learning with the less-affected forelimb after unilateral sensorimotor cortex lesions in rats. Eur. J. Neurosci. 22, 2069–2080.

Hsu, J.E., Jones, T.A., 2006. Contralesional neural plasticity and functional changes in the less-affected forelimb after large and small cortical infarcts in rats. Exp. Neurol. 201, 479–494.

Huie, J.R., Baumbauer, K.M., Lee, K.H., Bresnahan, J.C., Beattie, M.S., Ferguson, A.R., Grau, J.W., 2012. Glial Tumor Necrosis Factor Alpha (TNFalpha) Generates Metaplastic Inhibition of Spinal Learning. PLoS One 7, e39751.

Igarashi, T., Potts, M.B., Noble-Haeusslein, L.J., 2007. Injury severity determines Purkinje cell loss and microglial activation in the cerebellum after cortical contusion injury. Exp. Neurol. 203, 258–268.

Iida, H., Tachibana, S., Kitahara, T., Horiike, S., Ohwada, T., Fujii, K., 1999. Association of head trauma with cervical spine injury, spinal cord injury, or both. J. Trauma 46, 450–452.

Irvine, K.A., Ferguson, A.R., Mitchell, K.D., Beattie, S.B., Beattie, M.S., Bresnahan, J.C., 2010. A novel method for assessing proximal and distal forelimb function in the rat: the Irvine, Beatties and Bresnahan (IBB) forelimb scale. J. Vis. Exp. 46 (ID 2246).

Jones, T.A., Kleim, J.A., Greenough, W.T., 1996. Synaptogenesis and dendritic growth in the cortex opposite unilateral sensorimotor cortex damage in adult rats: a quantitative electron microscopic examination. Brain Res. 733, 142–148.

Jones, T.A., Allred, R.P., Adkins, D.L., Hsu, J.E., O'Bryant, A., Maldonado, M.A., 2009. Remodeling the brain with behavioral experience after stroke. Stroke 40, S136–S138.

Jones, T.A., Liput, D.J., Maresh, E.L., Donlan, N., Parikh, T.J., Marlowe, D., Kozlowski, D.A., 2012. Use-dependent dendritic regrowth is limited after unilateral controlled cortical impact to the forelimb sensorimotor cortex. J. Neurotrauma 29, 1455–1468.

Keppel, G., Wickens, T.D., 2004. Design and Analysis, A Researcher's Handbook, 4th edition. Pearson, Prentice Hallm Upper Saddle River, New Jersey.

Kokotilo, K.J., Eng, J.J., Curt, A., 2009. Reorganization and preservation of motor control of the brain in spinal cord injury: a systematic review. J. Neurotrauma 26, 2113–2126.

Lu, D.C., Zador, Z., Yao, J., Fazlollahi, F., Manley, G.T., 2013. Aquaporin-4 Reduces Post-Traumatic Seizure Susceptibility by Promoting Astrocytic Glial Scar Formation in Mice. J. Neurotrauma. http://dx.doi.org/10.1089/neu 2011.2114 (in press).

Luke, L.M., Allred, R.P., Jones, T.A., 2004. Unilateral ischemic sensorimotor cortical damage induces contralesional synaptogenesis and enhances skilled reaching with the ipsilateral forelimb in adult male rats. Synapse 54, 187–199.

Macciocchi, S.N., Bowman, B., Coker, J., Apple, D., Leslie, D., 2004. Effect of co-morbid traumatic brain injury on functional outcome of persons with spinal cord injuries. Am. J. Phys. Med. Rehabil. 83, 22–26.

Macciocchi, S., Seel, R.T., Thompson, N., Byams, R., Bowman, B., 2008. Spinal cord injury and co-occurring traumatic brain injury: assessment and incidence. Arch. Phys. Med. Rehabil. 89, 1350–1357.

Martinez, M., Brezun, J.M., Bonnier, L., Xerri, C., 2009. A new rating scale for open-field evaluation of behavioral recovery after cervical spinal cord injury in rats. J. Neurotrauma 26, 1043–1053.

McKenna, J.E., Prusky, G.T., Whishaw, I.Q., 2000. Cervical motoneuron topography reflects the proximodistal organization of muscles and movements of the rat forelimb: a retrograde carbocyanine dye analysis. J. Comp. Neurol. 419, 286–296.

Michael, D.B., Guyot, D.R., Darmody, W.R., 1989. Coincidence of head and cervical spine injury. J. Neurotrauma 6, 177–189.

Mihai, G., Nout, Y.S., Tovar, C.A., Miller, B.A., Schmalbrock, P., Bresnahan, J.C., Beattie, M.S., 2008. Longitudinal comparison of two severities of unilateral cervical spinal cord injury using magnetic resonance imaging in rats. J. Neurotrauma 25, 1–18.

Nishimura, Y., Isa, T., 2009. Compensatory changes at the cerebral cortical level after spinal cord injury. Neuroscientist 15, 436–444.

Nishimura, Y., Isa, T., 2012. Cortical and subcortical compensatory mechanisms after spinal cord injury in monkeys. Exp. Neurol. 235, 152–161.

Nout, Y.S., Mihai, G., Tovar, C.A., Schmalbrock, P., Bresnahan, J.C., Beattie, M.S., 2009. Hypertonic saline attenuates cord swelling and edema in experimental spinal cord injury: a study utilizing magnetic resonance imaging. Crit. Care Med. 37, 2160–2166.

O'Malley, K.F., Ross, S.E., 1988. The incidence of injury to the cervical spine in patients with craniocerebral injury. J. Trauma 28, 1476–1478.

Povolny, M., Kaplan, S., 1993. Traumatic brain injury occurring with spinal cord injury: significance for rehabilitation. J. Rehabil. Med. 59, 23–28.

Richards, J.S., Osuna, F.J., Jaworski, T.M., Novack, T.A., Leli, D.A., Boll, T.J., 1991. The effectiveness of different methods of defining traumatic brain injury in predicting postdischarge adjustment in a spinal cord injury population. Arch. Phys. Med. Rehabil. 72, 275–279.

Ricker, J.H., Regan, T., 1999. Neuropsychological and psychological factors in acute rehabilitation of individuals with both spinal cord injury and traumatic brain injury. Top. Spinal Cord Inj. Rehabil. 5, 76–82.

Rosenzweig, E.S., Courtine, G., Jindrich, D.L., Brock, J.H., Ferguson, A.R., Strand, S.C., Nout, Y.S., Roy, R.R., Miller, D.M., Beattie, M.S., Havton, L.A., Bresnahan, J.C., Edgerton, V.R., Tuszynski, M.H., 2010. Extensive spontaneous plasticity of corticospinal projections after primate spinal cord injury. Nat. Neurosci. 13, 1505–1510.

Schallert, T., Woodlee, M.T., 2003. Brain-dependent movements and cerebral-spinal connections: key targets of cellular and behavioral enrichment in CNS injury models. J. Rehabil. Res. Dev. 40, 9–17.

Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., Bland, S.T., 2000. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, Parkinsonism and spinal cord injury. Neuropharmacology 39, 777–787.

Scheff, S.W., Rabchevsky, A.G., Fugaccia, I., Main, J.A., Lumpp Jr., J.E., 2003. Experimental modeling of spinal cord injury: characterization of a force-defined injury device. J. Neurotrauma 20, 179–193.

Soicher, E., Demetriades, D., 1991. Cervical spine injuries in patients with head injuries. Br. J. Surg. 78, 1013–1014.

Sommer, J.L., Witkiewicz, P.M., 2004. The therapeutic challenges of dual diagnosis: TBI/SCI. Brain Inj. 18, 1297–1308.

Sprague, J.M., 1966. Interaction of cortex and superior colliculus in mediation of visually guided behavior in the cat. Science 153, 1544–1547.

Stambrook, M., Moore, A.D., Peters, L.C., Zubek, E., McBeath, S., Friesen, I.C., 1991. Head injury and spinal cord injury: differential effects on psychosocial functioning. J. Clin. Exp. Neuropsychol. 13, 521–530.
Strich, S.J., 1970. Lesions in the cerebral hemispheres after blunt head injury. J. Clin. Pathol. Suppl. (R. Coll. Pathol.) 4, 166–171.
Strong, M.K., Blanco, J.E., Anderson, K.D., Lewandowski, G., Steward, O., 2009. An investigation of the cortical control of forepaw gripping after cervical hemisection injuries in rats. Exp. Neurol. 217, 96–107.
Tian, H.L., Guo, Y., Hu, J., Rong, B.Y., Wang, G., Gao, W.W., Chen, S.W., Chen, H., 2009. Clinical characterization of comatose patients with cervical spine injury and traumatic brain injury. J. Trauma 67, 1305–1310.
Watanabe, T., Zafonte, R., Lairson, E., 1999. Traumatic brain injury associated with acute spinal cord injury: risk factors, evaluation, and outcomes. Top. Spinal Cord Inj. Rehabil. 5, 83–90.
Wei, C.W., Tharmakulasingam, J., Crawley, A., Kideckel, D.M., Mikulis, D.J., Bradbury, C.L., Green, R.E., 2008. Use of diffusion-tensor imaging in traumatic spinal cord injury to identify concomitant traumatic brain injury. Arch. Phys. Med. Rehabil. 89, S85–S91.
Willis, J.K., Morello, A., Davie, A., Rice, J.C., Bennett, J.T., 2002. Forced use treatment of childhood hemiparesis. Pediatrics 110, 94–96.
Wolf, S.L., Lecraw, D.E., Barton, L.A., Jann, B.B., 1989. Forced use of hemiplegic upper extremities to reverse the effect of learned nonuse among chronic stroke and head-injured patients. Exp. Neurol. 104, 125–132.